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SURGERY OF SUBPLEURAL BLEBS[1]

Indications and Contraindications

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Introduction

In 1945 Nissen (1) reported the conservative resection of a giant subpleural bleb. Since then there have been several similar reports (2–9). The present studies are part of an attempt to standardize the indications as well as the contraindications to this procedure.

Most writers use the terms *bleb* and *bulla* interchangeably. Miller (10, 11) defined a *bleb* as a thin-walled, bladder-like prominence found on the surface of the lung. He believed it to be due to the escape of air from a ruptured alveolus into the areolar layer of the visceral pleura, dissecting it from the underlying alveoli and ballooning out above the surrounding pleural surface. On the other hand, a *bulla* was regarded as the result of a localization of the change found in generalized emphysema. Interalveolar walls were destroyed by overdistention, forming a large air space within the lung tissue itself. This space communicated with a bronchiole. The pleura over a bulla remains intact, although increasing distention within the bulla might cause it to project beyond the level of the surrounding pleura. As will become evident in the following discussion, most of the surgically treated lesions are blebs.

These air spaces appear on a chest roentgenogram as radiolucent areas of varying sizes. They may consist of a single cavity or may be multilocular, as manifested by fine lines traversing them at right angles to the bronchovascular markings. Frequently the roentgenographic appearance is misleading. An apical bleb found at operation may not be visible on the chest film (6); or operation for what was believed to be a single large space may reveal multiple blebs and bullae, some large and some small. Occasionally the space may expand enough to show a concave border of compressed lung tissue (6). This condition is differentiated from pneumothorax by the absence of lung border at the periphery. If the pneumothorax is complete, the compressed lung will appear as a convex mass at the hilum. A large air cyst will cause a concave indentation of the remaining lung, which is usually not at the hilum.

Blebs rarely are infected (1, 4), and adhesions to the parietal pleura are minimal (1). The walls consist of thin, smooth membrane. The inner surface is shiny; the outer, dull and lusterless. It is usually 1 or 2 mm. in thickness. Microscopically the wall is fibrous connective tissue. There are occasional foci of lymphocytic infiltration. The inner fibers are hyalinized.

Blebs and bullae may cause no symptoms and be discovered by accident on routine chest roentgenography. The first evidence of air-space disorder in some patients is spontaneous pneumothorax due to rupture of a bleb (7); or gradually enlarging blebs and bullae, in one lung or both, may so compress adjacent alveoli as to cause increasing dyspnea. Baldwin and associates (12) divide these cases into three groups. Group 1 consists of large air cysts communicating freely with the bronchial tree. In Group 2 there is poor or intermittent bronchial communication, and the remaining portions of the lungs are normal. In Group 3*a* not only is the communication poor, but chronic diffuse emphysema is present with

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ventilatory insufficiency. In Group 3*b* alveolo-respiratory as well as ventilatory insufficiency exists. The symptoms of pulmonary insufficiency may or may not include cough, wheeze, sputum, cyanosis, et cetera. Hemorrhage and infection in bullae are rare but have been reported (13).

The present report is concerned with two groups of patients: the first consists of those who, for various reasons, have been subjected to operation for subpleural blebs. There are 3 such patients. The second group is made up of 4 patients presenting varied contraindications to operation.

Methods

All of the patients were studied by determination of the vital capacity and maximal breathing capacity by means of a 9-liter Collins respirometer, a modification of the Benedict-Roth metabolism apparatus (14). Bronchospirometry was performed using the Carlens catheter (15), recording on a Gebauer twin spirometer (16). Comparison was made with the normal values as given by Baldwin and associates (17).

Unless contraindicated by extreme pulmonary insufficiency, all patients considered for operation were studied by bronchoscopy and bronchography. In one case, angiocardiography was performed. This demonstrated the relative avascularity of the bullous area.

Observations

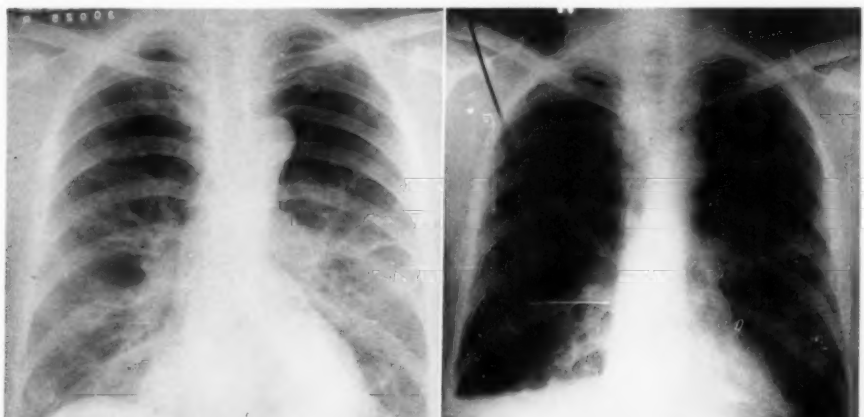
Situations in Which Operation Was Believed Advisable

Case 1. J. F. A. was a 62-year-old white man admitted to the hospital because of sudden onset of pain in the right chest, with dyspnea. He had suffered from "bronchial trouble" for more than fifteen years and noted slowly increasing dyspnea when walking up an incline. For three weeks prior to admission, he had noted a troublesome cough productive of small amounts of white sputum. He had smoked cigarettes for twenty years but had stopped two and one-half years before the onset of the present illness.

Physical examination on admission revealed a well-developed, moderately thin, wiry, white man who was dyspneic at rest. There was no cyanosis. The fingers were not clubbed. His chest moved minimally on both sides. He breathed with evident air-trapping. Both sides of his chest were hyperresonant, and breath sounds were almost inaudible except at the bases posteriorly.

A roentgenogram of the chest in the Veterans Administration regional office almost two years previously (figure 1) showed beginning development of an abnormal air space at the left apex. A chest film at the time of the present admission (figure 2) revealed a pneumothorax with 60 per cent collapse of the right lung and an increase in size of the left apical bleb.

A fenestrated catheter was placed into the right pleural cavity through the second intercostal space anteriorly and attached to an underwater



Case 1

Fig. 1 (Left). Roentgenogram obtained two years preoperatively.
Fig. 2 (Right). Appearance of roentgenogram on admission.

seal. The right lung re-expanded immediately, and the tube was removed four days later.

The patient was then submitted to studies designed to clarify the question of operation. Bronchoscopy revealed no abnormalities. Fluoroscopy revealed good aeration of the left lower lobe and the entire right lung. Both leaves of the diaphragm moved well, more on the left than on the right. No change in radiolucency of the left apex was noted on deep breathing. Paradoxical motion of neither the mediastinum nor the diaphragm was seen. Vital capacity (table 1) was somewhat more than the predicted value, while maximal breathing capacity was normal. Bronchospirometry demonstrated decreased ventilation, as well as decreased oxygen consumption by the left lung. Bronchography (figure 3) demonstrated compression of the remaining lung by the enlarged apical bleb, as manifested by crowding of the bronchi at the base.

At operation five large blebs up to 10 cm. in maximal diameter were seen: four in the left upper lobe and one in the anterior portion of the left lower lobe. There were only a few scanty adhesions at the apex. Many small bullae up to 1 cm. in diameter were scattered within the substance of the remaining lung.

The postoperative course was complicated by difficulty in bringing up mucus, mediastinal shift, and one episode of atrial flutter. By the end of the first week the patient was out of danger. The wound healed well, and the patient claimed to be subjectively much improved (figure 4). Bronchospirometry was repeated four months after operation (table 1). At this time the left was found to be the better lung. (The decrease in absolute values is due to the fact that in the postoperative determination the patient was less anxious and did not hyperventilate.) Unfortunately, subjective improvement was not mirrored in the maximal breathing capacity, which declined somewhat at first and then rose in subsequent determinations, but had not reached the preoperative value six months later. Postoperative decrease in ventilatory efficiency has been explained as due to further progress of emphysematous changes in the re-expanded lung (19). This theory would seem to be confirmed in this patient's case by the increasing vital capacity. In fact, bullae seem to be enlarging in the right midlung field (figure 4).

TABLE 1

PULMONARY FUNCTION STUDIES IN CASE 1

	Vital Capacity	Maximal Breathing Capacity
Predicted	3.4 liters	90.6 liters/min.
Preoperative	4.0 (120%)	84 (93%)
4 months postoperatively	3.7 (110%)	56 (62%)
7 months postoperatively	3.7 (110%)	68 (75%)
13 months postoperatively	4.4 (130%)	68 (75%)

Bronchospirometry

	Preoperative		Postoperative	
	Right	Left	Right	Left
Oxygen uptake (cc. per min.)	208 (82%)	42 (18%)	150 (38%)	250 (62%)
Tidal volume (cc.)	250 (62%)	150 (38%)	150 (60%)	100 (40%)
Vital capacity (liters)	—	—	1.10 (54%)	0.95 (46%)

Comment: Operation was performed on this patient in order to provide the reserve necessary to withstand another collapse or pneumonia in the right lung. It is believed that the operation accomplished the equivalent of giving the major portion of a new lung to a patient handicapped by slowly progressing pulmonary insufficiency. His outlook untreated was considered worse because he had suffered one major episode of acute pulmonary collapse. Follow-up examination will determine the necessity for operation on the right side.

Case 2. G. L. V. was a 54-year-old white man who complained of slowly increasing dyspnea of eight years' duration. In the three months before admission to the hospital, his condition became so much worse that he would have to stop several times to catch his breath while walking one city block. He also suffered from a productive cough and wheezing. Coincident with the deterioration in his pulmonary reserve, he noted mild left pectoral and scapular pain. A chest film in 1942, on induction into the army, was said to have been normal, but could not be obtained for comparison. The patient smoked one package of cigarettes daily until eight years before admission, when his symptoms of dyspnea and wheezing began.

On examination the patient was found to be a well-developed, muscular, white man of fifty-four. He was comfortable at rest. There was slight cyanosis of the lips and fingernails. The fingers were clubbed. The chest was emphysematous, and breath sounds were distant throughout.

A roentgenogram of the chest (figure 5) revealed bullous emphysema of the left lung. An electrocardiogram was consistent with chronic pulmonary disease. On fluoroscopy both sides of the diaphragm were found to move equally although

Case 1

FIG. 3 (Left). Bronchogram of patient.
FIG. 4 (Right). Postoperative roentgenogram.

Case 2

FIG. 5 (Left). Roentgenogram on admission.
FIG. 6 (Right). Bronchogram of left lung.

with somewhat diminished excursions. There was good aeration of the right lung. Aeration was poor on the left. There was slight paradoxical movement of the mediastinum toward the left upper and middle lung fields on inspiration.

Vital capacity (table 2) was 74 per cent of normal. Maximal breathing capacity was markedly diminished. Bronchospirometry revealed greater ventilation on the right, but equal oxygen consumption on both sides. Further studies at the East Orange, New Jersey, Veterans Administration Hospital revealed a large residual volume with an increased index of alveolar mixing. This would explain the apparently equal oxygen uptake

TABLE 2

CARDIOPULMONARY FUNCTION STUDIES IN CASE 2

	Vital Capacity	Maximal Breathing Capacity
Predicted	3.4 liters	90.6 liters/min.
Preoperative	2.71 (74%)	39.2 (43.5%)
6 months postoperatively	3.76 (110%)	73.0 (80%)

Preoperative Bronchospirometry

	Right	Left
Oxygen uptake (cc. per min)	300 (50%)	300 (50%)
Tidal volume (cc.)	300 (66%)	150 (34%)
Vital capacity (cc.)	650 (68%)	300 (32%)

Preoperative Studies at East Orange Veterans Administration Hospital

Total lung capacity	5.21 liters
Vital capacity	2.71
Expiratory reserve volume	1.08
Functional residual capacity	3.58
Residual volume	2.50
% residual volume/total lung capacity	48
Index of alveolar mixing	11.0% N_2

Arterial Blood	Breathing Air	Breathing Oxygen
O_2 saturation	94.4%	100%
CO_2 content	40.5 vol. %	41 vol. %

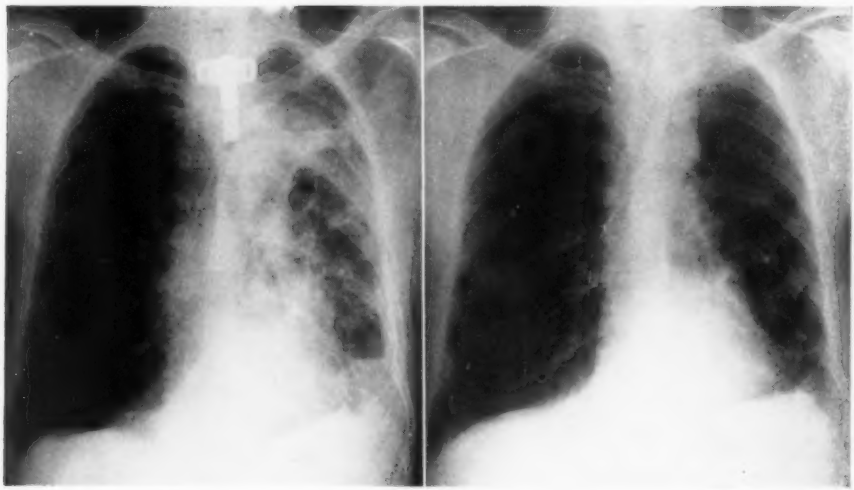
Cardiac Catheterization

	Volume Per Cent O_2	Pressure Mm./Hg.	Mean Pressure
At rest pulmonary artery	74.1	33/10	20
At rest systemic artery	95.3	120/60	105
Exercise pulmonary artery	47.3	57/20	35
Exercise systemic artery	91.4	135/85	120
Recovery pulmonary artery	66.7	33/10	20
Recovery systemic artery	94.4	140/75	100
Oxygen breathing pulmonary artery	72.6	25/10	15
Oxygen breathing systemic artery	99.0	140/80	110

by both lungs as due to trapping within the abnormal air spaces. This corresponds to some of the cases in Groups 2 and 3*a* of Baldwin and associates (12). Cardiac catheterization showed slightly increased pulmonary arterial pressure at rest, rising markedly with exercise. These data are to be expected with chronic pulmonary disease (18).

At bronchoscopy the findings were normal. Bronchography (figure 6) revealed compression of normal bronchi by the giant air space.

At operation the left lung, and particularly its upper lobe, was almost completely replaced by large and small blebs and bullae. The small blebs were mostly at the hilum and in the left lower lobe. These were not disturbed. Six large blebs, each 10 to 15 cm. in diameter, were found in the upper lobe. They had no communication with each other, as demonstrated by the fact that after resection of each succeeding bleb the rest remained inflated. Immediately after closure of the chest, an elective tracheotomy was performed to facilitate aspiration of secretions during the postoperative period. The left lung slowly re-expanded by creeping pleural symphysis (figures 7, 8), the mediastinum shifted to the left to compensate for loss of visceral space, and a hilar bleb expanded substernally. In spite of this difficult re-expansion, the patient, two months later on a follow-up visit, volunteered the information that he noted much less shortness of breath and now could walk up two flights of stairs with only slight discomfort. Six months



Case 2

FIG. 7 (Left). Roentgenogram two weeks after operation.
FIG. 8 (Right). Roentgenogram six months after operation.

postoperatively both his vital capacity and maximal breathing capacity (*see* table 2) showed marked improvement. He then returned to heavy manual labor without complaint.

Comment: The operative indication was actual pulmonary insufficiency due to compression of alveoli capable of functioning. The result was excellent. Because good pulmonary compliance was present, the operation was successful despite advanced emphysema. Even a history of right heart failure is not a contraindication to operation. On the contrary, in selected cases it prevents recurrence of the failure (19). Exactly how long improvement will last must be determined by continuing observation. In a few cases reported, the early gains have been temporary (20).

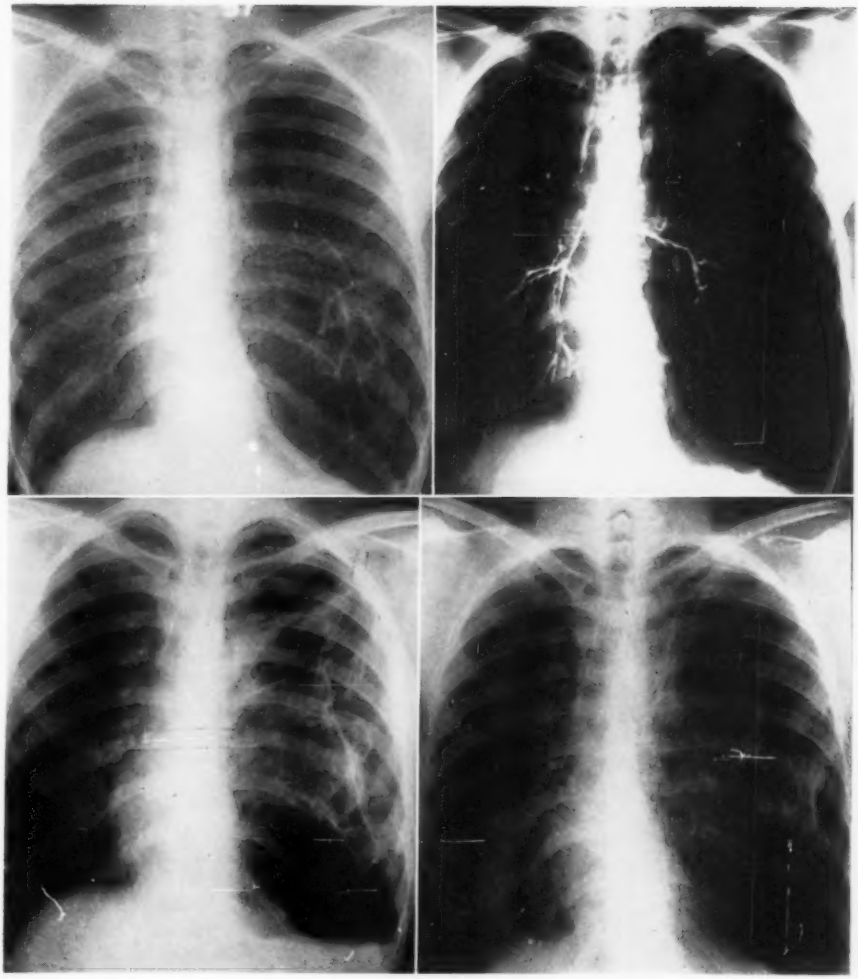
Case 3. J. E. Z. was a 25-year-old white man referred to the hospital because of an abnormal chest film noted by a mobile X-ray unit two months before admission. He suffered from frequent "colds" as often as every other month. These were manifested by congested nares. For the previous two years, he had noted slight traces of blood brought up by infrequent coughing. He had smoked more than a package of cigarettes daily for five years. He brought up a small amount of thick sputum on arising in the morning. He inhaled sawdust while at work as a carpenter.

Physical examination revealed a tall, thin, young man who appeared to be in excellent health. There was no finger clubbing. Auscultation of the chest revealed diminished breath sounds on the left.

A chest roentgenogram on induction into the army five years previously was normal. Three years later beginning emphysematous changes were seen at the left apex. A roentgenogram at the time of the present admission (figure 9) showed extensive bullous degeneration of the left lung. The apparent slight dextrocardia was present in all previous films, demonstrating that it was not due to increased tension in the air cysts. The findings at bronchoscopy were normal. On fluoroscopy of the chest, the entire diaphragm was seen to move normally. There was no paradoxical motion of the mediastinum. The right lung aerated well. The left lung was more radiolucent, but did not aerate as well on inspiration as the right. An electrocardiogram was normal.

Pulmonary function tests (table 3) were essentially normal for both lungs combined, but bronchospirometry demonstrated that all except an infinitesimal amount of ventilation, as well as oxygen uptake, were performed by the right lung. Bronchography (figure 10) showed compression of a normal left bronchial tree.

At operation the left upper lobe was found to be collapsed into the anterior part of the apex of the pleural cavity. Several small blebs were found in the upper lobe, none more than 2 cm. in diameter. The left lower lobe was a mass of large blebs up to 10 cm. in diameter. These did not communicate with each other, a finding similar to that in the preceding case. After unroofing these blebs, the underlying lung was found to be a spongy mass of bullae 1 to 2 cm. in diameter.



Case 3

FIG. 9 (Upper left). Preoperative roentgenogram.
FIG. 10 (Upper right). Bronchogram.
FIG. 11 (Lower left). Roentgenogram twelve days after operation.
FIG. 12 (Lower right). Roentgenogram six months after operation.

These could not be unroofed because they were completely surrounded by normal alveoli.

Postoperatively, apical and basal intrapleural air spaces (figure 11) had to be decompressed by repeated intercostal intubation. Healing was then complete (figure 12).

At a return visit six weeks later, the patient volunteered the information that his "wind" was now better, even though he had noted no shortness of breath previously. He claimed that now he could feel much more air entering his left lung. These subjective results were confirmed by a slightly increased maximal breathing capacity as well as by a marked increase in both tidal ventilation and oxygen uptake by the left lung (*see* table 3).

It will be noted (figure 12) that some bullous disease persists in the left lung. This was antici-

TABLE 3

PULMONARY FUNCTION STUDIES IN CASE 3

	Vital Capacity	Maximal Breathing Capacity
Predicted	4.0 liters	126 liters/min.
Preoperative	3.6 (90%)	92 (74%)
4 months postoperatively	3.6 (90%)	101 (80%)

Bronchospirometry

	Preoperative		4 Months Postoperative	
	Right	Left	Right	Left
Oxygen uptake (cc. per min.)	235 (94%)	15 (6%)	300 (67%)	150 (33%)
Tidal volume (cc.)	500 (77%)	150 (23%)	550 (69%)	250 (31%)
Vital capacity (liters)	1.8 (82%)	0.4 (18%)	2.3 (85%)	0.4 (15%)

pated at the time of operation. Because the small blebs in the upper lobe would merely expand to fill the pleural cavity postoperatively, resection of the lower lobe was not believed advisable.

Comment: By a comparatively simple operation, to be described in the next section, it is believed that this patient has regained the use of his previously almost functionless left lung. He is now in better condition to withstand any disease on the right side. Whether or not the degenerative process has been checked is a question which can only be answered by continuing observation.

Surgical Technique

The operation is performed in as simple a manner as possible to provide minimal risk. The patient lies flat on his back. General endotracheal anesthesia is used. The pleural cavity is entered through the fourth intercostal space anteriorly. Bleeding and operative time are much less than when the large posterolateral muscles are cut. If necessary, the fourth, third, or even the second costal cartilages can be cut for better exposure. All large blebs are unroofed. Bronchiolar openings, if seen, are separately sutured. Otherwise, the cut edges of the blebs are approximated over the raw, re-expanded lung. The parietal pleura is rubbed lightly with dry gauze to provoke adhesive reaction. The object is to provide as much alveolo-respiratory surface as possible. Therefore, no resection of lung tissue is planned.

If the degenerative process is absolutely confined to one lobe or segment (21, 22), resection would be indicated, but such a situation has not yet been encountered. Vagotomy or sympathetic denervation (21) have not been performed because pulmonary arterial pressure is reduced by bleb resection alone (19). It is believed that parietal pleurectomy (21) is an unnecessarily radical procedure. The reaction to dry-gauze rubbing is sufficient for pleural symphysis, as demonstrated particularly in the second case. Pleurectomy causes a painful stiffening of the chest wall for many weeks. This provides an added burden to the respiratory cripple. Every attempt to allow unhindered chest wall mechanics should be made. Increased pain on coughing threatens efficient tracheobronchial cleansing. Indeed, when respiratory reserve is minimal, the present writers recommend the elective performance of a temporary tracheotomy immediately on completion of the closure of the chest. Thus, aspiration of secretions is ensured.

Head and Avery (23) have suggested Monaldi intracavitary suction to decompress these abnormal air spaces. In the opinion of the writers, this multiple-stage procedure needlessly prolongs hospital stay. Its doubtful advantage is the use of local anesthesia throughout. As opposed to this is the fact that it is essentially a blind procedure. Well-managed endotracheal anesthesia actually supplies more oxygen to a patient with decreased pulmonary reserve than does room air breathed by a necessarily sedated patient operated upon under local anesthesia. Nor is this procedure a required compromise when both lungs are affected. Simultaneous bilateral thoracotomy is possible (22, 24). Again, the Monaldi procedure is effective only if the bleb is single or if all blebs intercommunicate. This proviso did not apply in 2 of the 3 surgical cases in the present series.

Situations in Which Operation Was Not Believed Advisable

Case 4. C. H. was a 61-year-old white man admitted because of gastric symptoms. A perforated duodenal ulcer had been repaired at another hospital twelve years previously. The patient had no pulmonary symptoms. A chest film on admission to the hospital (figure 13) demonstrated multiple small bullae throughout both lungs. His vital capacity was 3.9 liters, or 115 per cent of normal. The maximal breathing capacity was 62 liters per

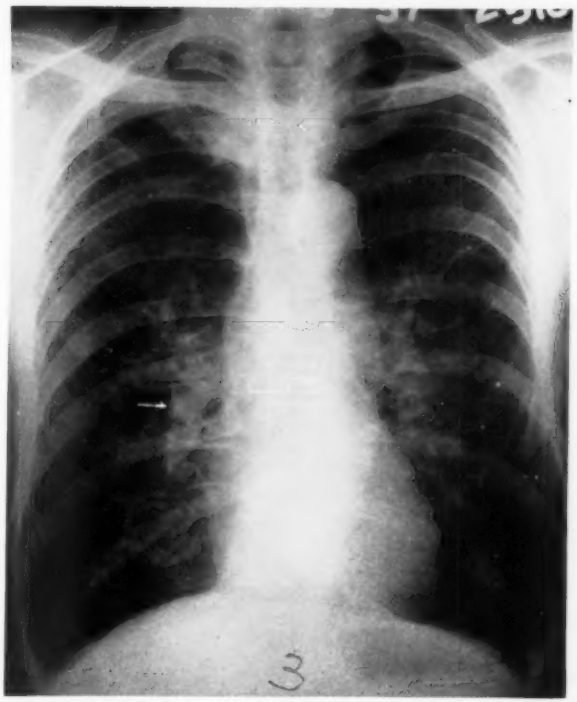


FIG. 13. *Case 4.* Roentgenogram demonstrating multiple small bullae throughout both lungs.

minute, or 69 per cent of normal; the air velocity index was 0.6.

Comment: This patient was asymptomatic. The bullae were not large enough to cause marked compression of adjacent alveoli. They were too diffuse for localized surgical therapy and were bilateral. Here, only further observation is necessary to watch for progression of the disease and enlargement of the air spaces. Caffey (25) has reported spontaneous return to normal of such air spaces in infants and children following recovery from respiratory infections. In fact, he has seen them enlarge and shrink with comparative rapidity. No such speedy evolution has been reported in adults.

Case 5. F. W. B. was a 68-year-old retired coal miner. He had been exposed to anthracite dust for fifteen years. For seven years before admission, he had experienced increasing dyspnea. He suffered occasional night sweats. About six months before admission, he had coughed up a small amount of blood. Since then he had been short of breath even at rest, and experienced two-pillow orthopnea.

On physical examination the patient appeared chronically ill. He was dyspneic sitting up in bed. There was slight cyanosis of the fingertips; they were moderately clubbed. The chest was emphysematous, and respiratory excursions were limited in extent. Percussion note was hyperresonant throughout. An occasional rhonchus was auscultated.

A chest roentgenogram (figure 14) showed generalized anthracosilicosis, third stage, bilateral. There was a large bulla in the left lower lung field. Hemoglobin was 17 gm. per 100 ml. The erythrocytes numbered 5,451,000 per cu.mm. Repeated sputum examinations failed to show acid-fast bacilli on either smear or culture. An electrocardiogram was normal.

The patient had episodes of low-grade fever and his sputum varied in amount. At bronchoscopy the tracheobronchial mucosa was found to be red and edematous. Within the lumen there was copious thick, purulent, brown to gray mucus.

Pulmonary function tests (table 4) demonstrated marked diminution of ventilatory efficiency. On bronchospirometric study, the right lung ventilated approximately twice as well as the

FIG. 14. *Case 5.* Roentgenogram demonstrating generalized anthracosilicosis, third stage, bilateral.

TABLE 4

PULMONARY FUNCTION STUDIES IN CASE 5

Vital capacity	1.35 (predicted 3.4 liters) 40% of predicted
Maximal breathing capacity	19.6 liters/min. (predicted 90.6) 22%

Bronchospirometry

	Right	Left
Oxygen consumption (cc./min.)	400 (61.5%)	250 (38.5%)
Tidal volume (cc.)	150 (67%)	75 (33%)
Vital capacity (cc.)	475 (70%)	200 (30%)

left. This finding was paralleled by the oxygen consumption on each side.

Comment: This patient's shortness of breath and anoxia were caused by three factors: (*1*) pulmonary fibrosis, diffuse; (*2*) chronic bronchitis with large amounts of pus partially obstructing the bronchi; and (*3*) a large bulla at the left base. Because the proportion of oxygen taken up by the left lung was only slightly below normal, it is believed that the first two factors were of the greater importance in this case. Adjacent alveoli were not sufficiently compressed to deprive the left lung of more than a small portion of its respiratory surface. Operative re-expansion would therefore not sufficiently improve this patient's anoxia to warrant the risk of surgery.

Kaltreider and Fray (26) attempted an operation on a coal miner with bilateral blebs. He died two hours later. Baldwin and her co-workers (12) list these cases in their Group 3*b*—"air cysts with poor or intermittent bronchial communication, associated with chronic diffuse emphysema in the remaining lungs; disability due to both ventilatory and alveolo-respiratory insufficiency." Of this subgroup, only two patients were subjected to surgery and both died. The writers described the operative findings as follows: (A large bulla) "was resected but when re-expansion of the remaining lung was attempted, innumerable other bullae were created, almost like soap bubbles. The patient died a few days later of pulmonary insufficiency." No roentgenograms were published with their paper, which was essentially a physiologic study. Nevertheless, it is likely that

these patients were anoxic because of pulmonary fibrosis and not alveolar compression. The lung had lost its compliance. Attempts at reinflation merely blew up more bullae.

The only death in Crenshaw and Rowles' (21) first series was of a patient with silicosis. They state that he died thirteen days after operation "because of persistent air leaks and partial lung collapse."

Dowell and associates (19) claim three successful results in patients they believe correspond with Baldwin's Group 3*b*. However, their roentgenographic studies show no fibrosis. It is believed that these patients belong in Group 3*a*, exhibiting only diffuse emphysema. The emphysematous lung still possesses satisfactory compliance (*Cases 2* and *3*); the fibrotic lung does not.

It is planned to attempt to settle this question by determinations of lung compliance, in addition to other studies.

Case 6. K. F. B. was a 22-year-old white man who complained of increasing shortness of breath, weakness, and weight loss of one year's duration. At the time of onset of symptoms, a chest film revealed diffuse bilateral pulmonary fibrosis. A biopsy of the left lung was performed at the Philadelphia Naval Hospital. A slide of the specimen was obtained, and the finding of a chronic nonspecific granuloma of the lung was confirmed. Microscopically there was increased fibrosis. The tissues were markedly infiltrated by eosinophils. No tubercles were seen. Special stains for acid-fast bacilli and fungi were negative.

Before admission to this hospital, the patient had had three episodes of pneumothorax, two on the left and one on the right side. These had re-expanded, but dyspnea increased. At the time of admission, he could not climb one flight of stairs at a normal pace.

Physical examination revealed an ill-developed, asthenic, sallow, dyspneic, young man. Breath sounds were decreased throughout both lungs. A chest roentgenogram on admission (figure 15) revealed extensive bilateral diffuse fibrosis with multiple small bullae throughout both lung fields. The vital capacity was 2.24 liters, or 56 per cent of normal. Maximal breathing capacity at first was 67 liters per minute, or 53 per cent of normal, but this determination was done before marked deterioration in the patient's condition.

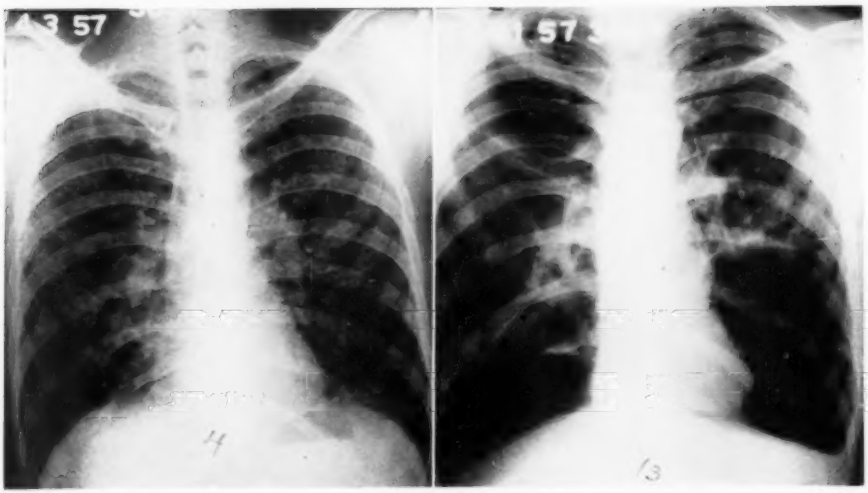
The patient was treated with bronchodilators, oxygen, and antimicrobial therapy. Prednisone therapy was started but was discontinued when a right pneumothorax developed. For one week the internist attempted to re-expand the right lung by repeated needle aspiration of pleural air. Then the surgeon was requested to insert an intercostal catheter attached to an underwater seal. Even with 25 cm. of water suction, re-expansion could not be accomplished. Repeated intubations of the pleural air space over the next twelve days failed to re-expand the contracted lung. When pleural symphysis was finally established, the fibrotic lung could not expand to fill the space. Instead, the bullae in both lungs rapidly enlarged (figure 16).

By this time the patient was *in extremis*, dyspneic even in an oxygen tent. The surgeon (F. F.) was requested to see this patient in consultation, but believed surgery not advisable. The patient died sixteen days after the giant bullae were first noted. Permission for post-mortem examination was refused.

Comment: Why was operation not done? Because the long course of unsuccessful intercostal intubations demonstrated lack of pulmonary compliance. When pleural symphysis was finally accomplished, it was at the expense of small bullae which rapidly enlarged to fill the pleural cavity. The lung itself could not expand homogeneously.

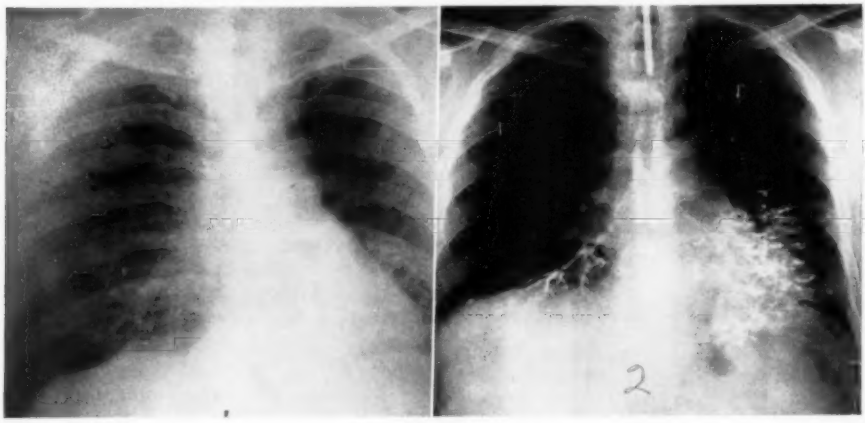
But even if the surgical attempt was foredoomed to failure, why not make the attempt and hope for a miracle—or at least a fallacy in the reasoning? Because it is believed that the procedure was actually contraindicated. Anyone who has watched at the bedside of a patient who has just undergone a pulmonary resection will remember the appearance of the drainage bottles. If controlled suction is applied to accelerate the re-expansion of the rest of the lung, there is bubbling of escaping air on both inspiration and expiration. Certainly, suctioning out a portion of this patient's inspired air would not diminish his anoxia. If no suction is attached to the drainage bottles, the bubbles appear only with expiration. But there is wide fluctuation of the fluid level in the chest tubes on inspiration. In other words, postoperative chest drainage tubing increases anatomic dead space. The volume of the lumen of each tube from chest wall to water-trap is 40 cc. by direct measurement. Therefore, operation would have increased this patient's dead space by as much as 80 cc. This also would have been too much of a burden for this moribund, anoxic patient. His only hope lay in reversal of the fibrotic process by steroid therapy. Unfortunately, this was unsuccessful.

Case 7. S. S. L. was a 35-year-old white man who complained of left chest pain of five years' duration. On one occasion, four or five years before admission, he had coughed up a small amount of blood. His pain was worse in damp weather. At



Case 6

FIG. 15 (Left). Roentgenogram on admission.
FIG. 16 (Right). Roentgenogram one day ante mortem, when the question of emergency surgery was raised.



Case 7

FIG. 17 (Left). Roentgenogram revealing abnormal air space at the left apex.
FIG. 18 (Right). Bronchogram.

nine months of age he had had pneumonia, followed by empyema requiring surgical drainage.

The patient was a stocky, muscular, young man who appeared neither acutely nor chronically ill. There was no finger-clubbing. There were two old healed scars on the left lower chest posteriorly. The left chest moved less than the right.

A chest film (figure 17) revealed an abnormal air space at the left apex. An electrocardiogram showed a few premature ventricular contractions. No acid-fast bacilli or fungi were found in the sputum.

On fluoroscopy of the chest it was noted that the right lung aerated normally. Motion of the right half of the diaphragm was normal. On the left, the diaphragm was obscured by the general pleural haziness. It was difficult to note any left lower lobe aeration. The radiolucency in the

TABLE 5

PULMONARY FUNCTION STUDIES IN CASE 7

Vital capacity	3.7 liters (predicted 4.16) 89%
Maximal breathing capacity	95 liters/min. (predicted 109.4) 87%

Bronchospirometry

	Right	Left
Oxygen consumption (cc./min.)	350 (87%)	50 (13%)
Tidal volume (cc.)	350 (58%)	250 (42%)
Vital capacity (cc.)	2,000 (78%)	550 (22%)

left upper lung field was unchanged during the respiratory cycle. The upper mediastinum moved toward the left on inspiration.

Pulmonary function tests (table 5) demonstrated almost normal ventilation. On bronchospirometry it was found that most of the oxygen uptake was by the right lung. Ventilation on the left was diminished to a somewhat less extent. A bronchogram of the left lung (figure 18) delineated extensive saccular bronchiectasis.

Comment: The only symptom was pain. This would not be alleviated by operation; it might even be increased. One occasion of hemoptysis in five years can be ignored. There were no symptoms of pulmonary infection. The left lung was partially destroyed and bronchiectatic. The abnormal air space at the left apex was of coincidental importance only. The sole procedure possible in this patient would be a pneumonectomy. Because some slight oxygen absorption took place in the left lung, its total excision could not improve his pulmonary reserve. It would be indicated only if the bronchiectatic cavities became infected. But surgery for infected bronchiectasis is not the subject of the present report.

The patient has been taught postural drainage as a daily exercise to avoid infection of stagnant mucus. He has been instructed to return in case of development of fever or pulmonary symptoms. He has had no complaints for the past twenty-one months.

SUMMARY

An attempt has been made to clarify the indications for the surgical resection of subpleural blebs. It is believed that the operation should be conservative of lung tissue. Only giant blebs are considered for removal. They must be big enough to cause demonstrable compression of adjacent lung tissue. The blebs must be the major factor in the altered respiratory efficiency. Coexistent diffuse emphysema may also be present but is probably part of the same process. However, any other added pulmonary disease should raise doubt of the primary importance of the blebs. Further studies, particularly of lung compliance, are indicated. The operation is recommended both as a prophylactic and a therapeutic measure to improve pulmonary function.

SUMARIO

La Cirugía de las Vesículas Pulmonares: Indicaciones y Contraindicaciones

Trátase de esclarecer las indicaciones para la resección quirúrgica de las vesículas subpleurales. Opínase que la operación debe tratar de conservar el tejido pulmonar. No hay que considerar para extirpación más que las vesículas grandes, de tamaño suficiente para ocasionar compresión demostrable del tejido pulmonar adyacente. Las vesículas deben constituir el factor mayor en la alteración de la eficacia respiratoria. Puede también haber un enfisema difuso coexistente, pero forma probablemente parte del mismo proceso. Sin embargo, cualquiera otra dolencia sobrepuesta debe engendrar dudas acerca de la importancia primordial de las vesículas. Hállanse indicados nuevos estudios, en particular de la acomodación pulmonar. Recomiéndase la operación como providencia tanto profiláctica como terapéutica para mejorar la función pulmonar.

RESUME

Chirurgie des bulles sous-pleurales: Indications et contre-indications

Il a été tenté de clarifier les indications de la résection chirurgicale des bulles sous-pleurales. Les auteurs estiment que dans cette intervention il y a lieu de conserver la plus grande quantité possible du parenchyme pulmonaire. Seules les bulles géantes sont considérées justiciables de l'exérèse. Elles doivent être assez importantes pour comprimer notablement le parenchyme adjacent. Les bulles doivent être le facteur majeur dans l'altération de la fonction respiratoire. L'emphysème diffus coexistant peut être également présent, étant probablement partie intégrante du même processus. Toute autre affection pulmonaire surajoutée doit faire hésiter à donner aux bulles une importance primordiale. Il y lieu de poursuivre les études sur les variations du volume pulmonaire sous l'influence des différences de pression. L'intervention chirurgicale est recommandée à la fois comme mesure préventive et curative pour améliorer la fonction pulmonaire.

Acknowledgments

The writers are grateful to Dr. George Leiner, Dr. Henry Green, and Mr. Sol Abramovitz of the East Orange, New Jersey, Veterans Administration Hospital for the cardiopulmonary study of *Case 2*. Mr. Gilbert E. Noll and Mr. Z. J. Savitski assisted in the studies at Wilkes-Barre.

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THE DISTINCTION BETWEEN REGENERATIVE AND ATYPICAL ALTERATIONS IN THE BRONCHIAL MUCOSA[1]

DAVID M. SPAIN

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Introduction

The recognition and proper interpretation of early alterations in the bronchial epithelial lining are of the utmost importance in the evaluation of the significance of various factors in the causation of bronchogenic carcinoma. In 1951, Spain and Parsonnet (1) reported a case with multiple primary bronchogenic carcinomas. The patient exhibited a variety of noninvasive atypical alterations in widely scattered areas in the bronchial mucosa of both lungs. Some of these alterations had sufficient absence of cellular differentiation, disorganization, and variation in nuclear structure and size so that they could be interpreted as carcinoma *in situ*. Others have since reported similar observations (2–4). Of pertinence to this report is the fact that none of these noninvasive but otherwise atypical changes resembled any of the alterations seen in the various stages of progression of "normal" epithelium into squamous metaplasia that are so commonly observed in chronic inflammatory conditions of the lung.

Recently, Auerbach and associates (5), in a detailed study on the early alterations in the bronchial mucosa as related to the smoking habit, reported on the comparative incidence of these various changes. Several confusing and disturbing features that are evident in the findings of this latter study have unfortunately tended to diminish the significance of its conclusions. Among these puzzling features is the observation of a number of cases of carcinoma *in situ* in the tracheal mucosa despite the fact that primary carcinoma of the trachea is exceedingly rare in this part of the world. Another is the finding of carcinoma *in situ* in as high an incidence as one of four non-cigarette-smoking males. This also is in sharp contrast to the relatively infrequent occurrence of bronchogenic carcinoma in the non-smoking male. Still another is the observation that these alterations revealed no predilection for the larger bronchi where the major sites for primary carcinoma are located. Then there was the implication that the lesions perhaps progressed through stages of basal cell hyperplasia, stratification, metaplasia, and into carcinoma *in situ*. Finally, no clear distinction was made between regenerative lesions that do not have the same biologic potential for malignant neoplasia and those atypical alterations that have an enhanced neoplastic potential.

In another recent report by Valentine (6) on early changes and carcinoma *in situ* of the bronchial mucosa, the biologic distinction between regenerative and atypical changes was also not clearly defined. The experience of most observers has been that such alterations as basal cell hyperplasia, stratification, and squamous metaplasia that usually occur as regenerative phenomena in such conditions as bronchiectasis or chronic organizing pneumonia are not associated with any special predilection for the development of bronchogenic carcinoma. For this reason and because of the various confusing reports, a restudy of the distribution of these alterations in the bronchial mucosa was undertaken.

Plan of Investigation

The problem was approached by reviewing available sections of the bronchial mucosa in 50 cases of chronic inflammatory pulmonary disease in males over the age of forty-five. These included 35 cases of bronchiectasis of long duration and a miscellaneous group of cases of chronic organizing pneumonias. This age group was deliberately selected so that it would correspond more closely to the age group of the patients with bronchogenic carcinoma in this study. Also reviewed were the epithelial changes in the bronchi of 50 males with bronchogenic carcinoma. The sections studied were those adjacent to the carcinoma and those from other major bronchi, as well as sections from the smaller ramifications of the bronchial tree. Five to ten bronchial sections were available in each case. Since no standard criteria were utilized in the taking of the sections, this study does not

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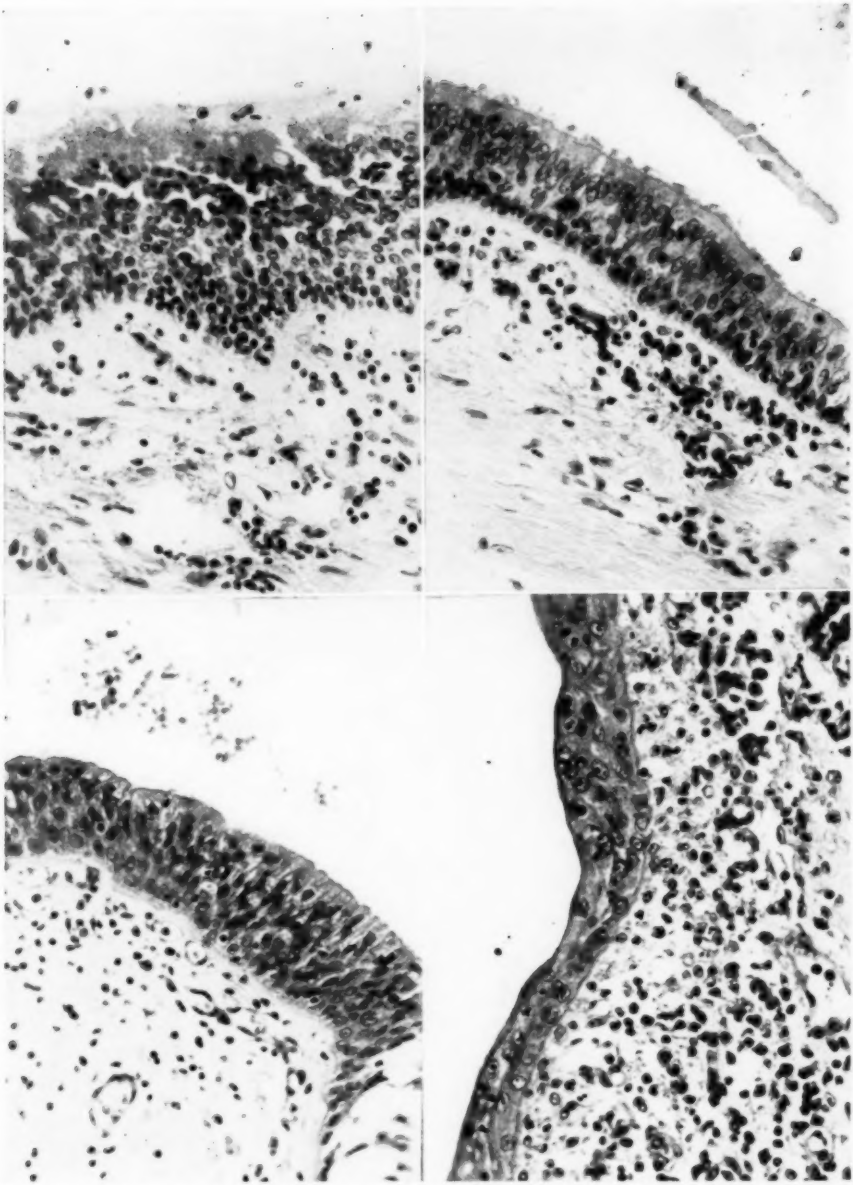

FIG. 1 (Upper left). Basal cell hyperplasia from a major bronchus in a case of bronchiectasis. (× 520)
FIG. 2 (Upper right). Basal hyperplasia from a minor bronchus in a case of chronic organizing pneumonia. (× 520)
FIG. 3 (Lower left). Stratification of bronchial mucosa in a case of bronchiectasis. (× 520)
FIG. 4 (Lower right). Squamous metaplasia in major bronchus in a case of chronic organizing pneumonia. (× 520)

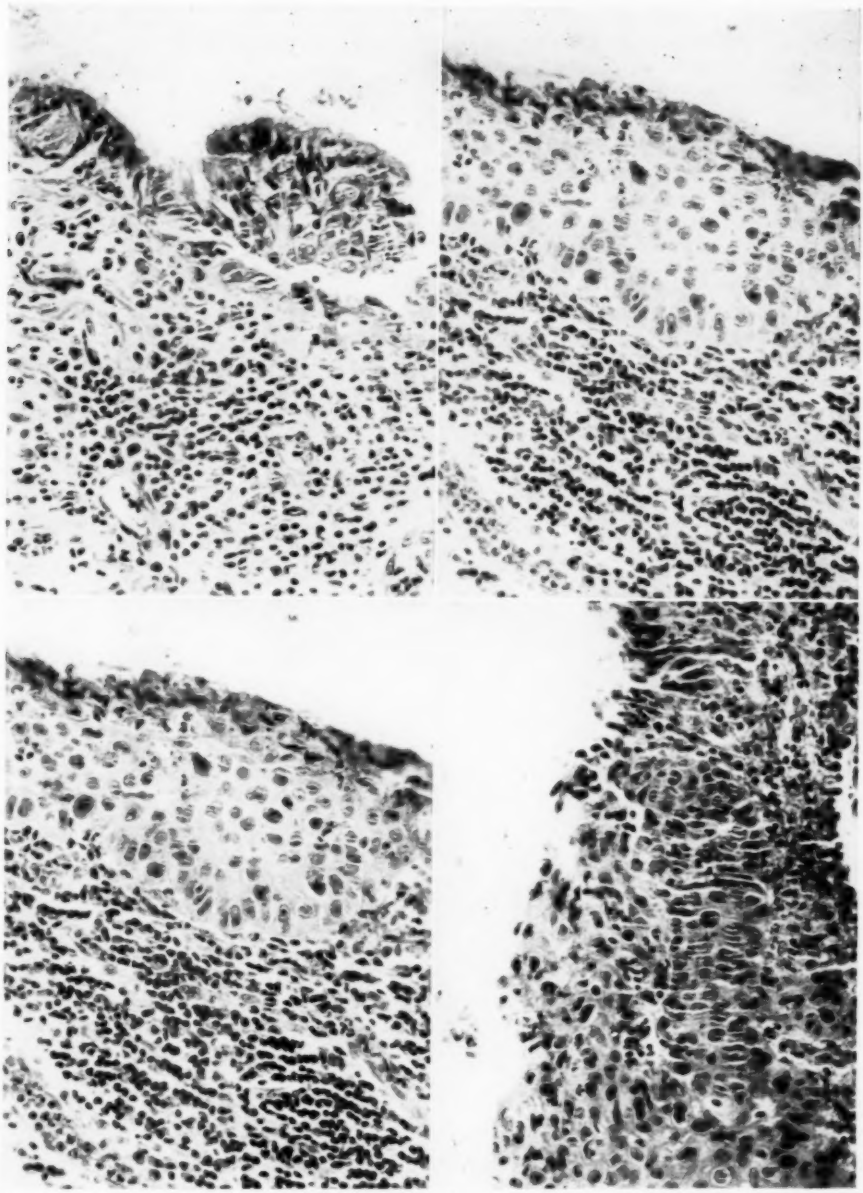

FIG. 5 (Upper left). Atypical alterations in major bronchus 2 cm. from site of primary carcinoma. (× 520)

FIG. 6 (Upper right). Hyperplastic changes with some atypical cells and nuclear alterations in mucosa of bronchus which was not the site of the primary tumor (× 520).

FIG. 7 (Lower left). Changes similar to those shown in figure 6 (taken from another case). (× 520)

FIG. 8 (Lower right). Carcinoma *in situ* in a major bronchus. Another major bronchus was involved with carcinoma. (× 520)

lend itself to any conclusions concerning the incidence or relative frequency of the various lesions. The same terminology employed in other studies was utilized for the classification of the lesions. This consisted of *basal cell hyperplasia, stratification, squamous metaplasia, atypical hyperplasia,* and *carcinoma in situ.* The criteria for atypical cells were: loss of polarity; disorganization of basal layer; numerous and abnormal mitoses; nuclear alterations in staining, size, shape, and chromatin; and the failure of cells to maturate, as well as the over-all extent of these changes. These are the criteria that have been established for the proper interpretation of atypical changes for carcinoma *in situ* in the uterine cervix, with which there has been more experience. The slides were coded so that at the time of original observation and interpretation it was not known in which of the two groups the slides belonged.

Observations

The findings may be briefly summarized. None of the sections from the inflammatory group of cases revealed any of the atypical changes described above. All of the cases in the inflammatory group revealed various degrees of involvement with either basal cell hyperplasia, stratification, or squamous metaplasia (figures 1–4). This study was not concerned with those atypical collections of spindle or undifferentiated cell proliferations that are occasionally observed, particularly in cases of bronchiectasis, adjacent to some of the smaller bronchioles. These, however, are generally regarded as benign.

In the cases of bronchogenic carcinoma, all revealed some of the regenerative changes already described and observed in the chronic inflammatory conditions of the lung. In addition, 21 of the 50 cases revealed in at least one section some atypical changes that were not in direct continuity or anatomic relationship with the bronchogenic carcinoma. These changes appeared as abrupt transitions in either partially metaplastic epithelium or in otherwise apparently unaltered epithelium. These varied from atypical hyperplasia to carcinoma *in situ.* These changes were confined entirely to the mucosa of the major bronchi (figures 5–8). The other alterations, how-

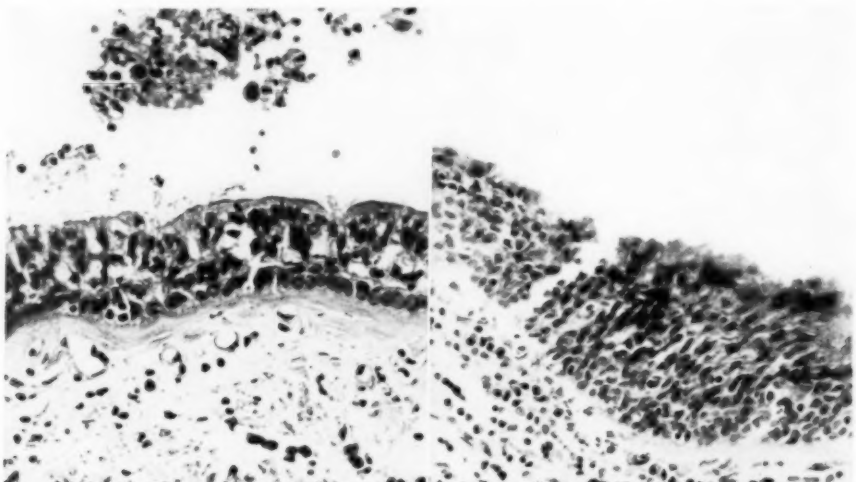

Fig. 9 (Left). Some alterations in cell shape, size, and nuclei in hyperplastic bronchial epithelium. (× 520)

Fig. 10 (Right). Marked proliferation of cells with failure of maturation near the surface in a case of bronchogenic carcinoma. (× 520)

ever, were frequently present in the smaller ramifications of the bronchial tree. Occasionally, changes were noted which were difficult to classify and were probably distorted by associated active inflammation (figures 9 and 10).

Discussion

The changes seen in the progression from a "normal" epithelial lining into carcinoma *in situ* have been best documented in cases involving the uterine cervix. In this region a clear distinction is generally made between squamous metaplasia (epidermatization) that is frequently present, and which is unrelated to the development of carcinoma, and those atypical changes that may progress into invasive carcinoma. This same distinction should be applied to similar changes in the bronchial mucosa. It is recognized that there is some evidence that chronic regenerative processes in lining epithelium on rare occasions may eventuate in carcinoma, i.e., squamous cell carcinoma that develops in a chronic-burn ulcer.

Whether or not squamous epithelium or metaplastic epithelium is less resistant to comparable doses and time exposure of carcinogens than other forms of epithelium is an entirely different problem, to which there is as yet no clear answer. In the bronchial tree, the ciliary activity and mucous production of the lining cells are important cleansing mechanisms for the removal of foreign particles. When metaplastic changes are present and ciliary activity and mucous secretion are lost, noxious agents or carcinogens may remain in more intimate contact with the lining cells for longer periods of time. In this sense, regenerative changes may perhaps enhance the possible development of bronchogenic carcinoma.

In this study, the cases of chronic inflammatory pulmonary disease revealed only regenerative changes despite their long duration and the older age of the patients. In the cases of bronchogenic carcinoma, both regenerative and atypical changes were present. The atypical changes may very well have been a response to the same carcinogenic factors that produced the bronchogenic carcinoma. The regenerative changes could very well be the response to the nonspecific irritating effects of the noxious agents. For instance, in cigarette smoke there could conceivably be both specific carcinogens and noncarcinogenic irritative substances.

Studies that attempt to formulate a correlation between the development of carcinoma and specific noxious agents should make careful distinction between the regenerative and the atypical alterations. Furthermore, rigid and accepted criteria should be applied in classifying lesions as carcinoma *in situ*. It is likely that the application of such criteria to previous or current studies may reveal an even more clear-cut association between cigarette smoking and atypical changes in the bronchial epithelium than has been reported heretofore.

Summary

A review of the changes in the bronchial mucosa in chronic inflammatory pulmonary disease and bronchogenic carcinoma reveals two apparently independent groups of alterations. One is a regenerative phenomenon which is not directly related to the development of carcinoma, and the other consists of atypical changes which include carcinoma *in situ* and which undoubtedly have the biologic potential for invasive neoplasia.

These two groups of changes do not appear to be biologically related to each other.

Attempts to correlate early alterations in the bronchial mucosa with possible carcinogens should take these distinctions into account.

Sumario

La Distinción entre las Alteraciones Regenerativas y Atípicas de la Mucosa Bronquial

Un estudio de las alteraciones de la mucosa bronquial observadas en la neumopatía inflamatoria crónica y en el carcinoma bronquiógeno revela dos grupos de alteraciones al parecer independientes. Uno es un fenómeno regenerativo y sin relación directa con la aparición del carcinoma, y el otro consiste en alteraciones atípicas que comprenden el carcinoma *in situ* y poseen indudablemente el potencial biológico para la invasión neoplásica.

Estos dos grupos de alteraciones no parecen hallarse relacionados biológicamente entre sí.

Los esfuerzos encaminados a correlacionar las alteraciones incipientes de la mucosa bronquial con los posibles carcinógenos deben tomar en cuenta estas distinciones.

Resume

La distinction entre les altérations atypiques et régénératives dans la muqueuse bronchique

Une analyse des modifications de la muqueuse bronchique dans la maladie pulmonaire inflammatoire chronique et le cancer bronchogène

révèle deux groupes d'altération indépendants. L'un est un phénomène de régénération, non directement en relation avec le développement du cancer et l'autre consiste en modifications atypiques, qui comportent le cancer *in situ*, et qui ont indubitablement le potentiel biologique de la néoplasie envahissante.

Ces deux groupes de lésions ne paraissent pas être biologiquement apparentés entre eux.

Il y a lieu de tenir compte de ces distinctions lorsqu'il s'agit d'établir une relation entre les altérations précoces de la muqueuse bronchique et la néoplasie possible.

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SEGMENTAL ATELECTASIS IN CHILDREN WITH PRIMARY TUBERCULOSIS[1]

SIMON FROSTAD

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Introduction

The first description of the primary complex was in 1876 by Parrot (1). He showed the close connection between the lung focus and the hilar lymph node disease, and described it as "miroir des poumons." He further said "il n'y a pas d'adénopathie bronchique qui n'ait d'une origine pulmonaire"—the well-known *law of Parrot*. Later writers have had little to add to the observations made by Parrot. Kuss (2) and Ghon (3–5), who published their works in 1898 and 1912, respectively twenty-two and thirty-six years later than Parrot, have confirmed the observations made by him.

The primary lung focus is often a small focus which, after an exudative phase during which a malignant development *can* occur, usually passes through a caseous phase, encircled by a zone of tubercles which in turn will be encircled by a fibrous capsule. Both the capsule and the caseous tissue will be inspissated with calcareous or bony tissue in the stage of healing. In children, the lymph node reaction is more distinct and extensive than the reaction in the primary focus. This has been shown both roentgenographically and histologically by Pagel (6) and other observers. The reaction in the lymph node component often continues for some time. Here also, in time, a stage of healing usually takes place in the form of a central caseous tissue encircled by a zone of tubercles and a fibrous capsule, finally inspissated with calcareous masses. Both the lung focus and the lymph node component in their latent caseous and calcareous stage may contain tubercle bacilli.

In some cases the contents of the lymph nodes will be converted into a purulent mass, forming a closed lymph node abscess. This happens rather frequently in smaller children, in whom both the primary focus and the lymph node component can be enormously enlarged. These enlarged lymph nodes can compress adjacent organs such as the bronchi and blood vessels, and may even discharge their contents into them. With sufficient bronchial compression there may be an atelectasis of the corresponding segments; and in a short period before the occlusion there can be a segmental emphysema. Compressed blood vessels are not obvious by the ordinary examination methods of today, but the consequences of penetration of the blood vessels may be observed in the form of hematogenous bacillary spread in the lung itself or to other organs.

Segmental atelectasis in children was first described in 1920 by Eliasberg and Neuland (7–9) who gave it the term epituberculosis. Simon and Redeker (10–12) later called it perifocal inflammation, but Rössle (13) in 1936 showed that epituberculosis in reality was atelectasis from compression of a bronchus by lymph nodes. He distinguished the pure atelectasis from the complicated cases which often show pneumonic foci as a result of aspiration of bacillary material discharged from the perforated lymph node. The adjacent lymph node presses the bronchus and closes the corresponding bronchial segments, which results in a pure atelectasis. If the enlarged lymph node is removed at this point, the involved lung segments in most cases will be filled with air, and there will be no pathologic alterations in the parenchyma of the lungs. This operation has been done by Dilwyn Thomas (14) and others. If, however, the atelectasis continues long enough, a nontuberculous infection will arise with development of fibrosis of the lung and ectasis of the bronchi peripheral to the bronchial stenosis. If the lymph node erodes the bronchial wall and empties its contents into the bronchial tree, the tuberculosis then may spread through the pathologic tissue. This occurs much more easily, as the previously blocked lung segments will now be filled with air. The bronchial stenosis often discontinues when the perforation of the lymph node occurs. As this air flow may pass a continual discharging fistula, often with a purulent fluid rich in tubercle bacilli, it is not difficult to imagine how the spread of this infectious material out to the corresponding lung segments takes place.

The present report is concerned with 90 cases of segmental atelectasis in children with primary

[1] From Grefsen Sanatorium, Oslo, Norway.

tuberculosis from the Childrens Department at Grefsen Sanatorium. There were 47 boys and 43 girls, ranging in age from eight months to fourteen years. All of the patients were seen in the last three to three and a half years. Apart from the age group of two to four years, in which there were 24 children, the other age groups showed fairly even distribution.

Observations

All of the children were bronchoscoped, most of them by Dr. Lars Hansen, but a few of the older ones by the writer. In all, 90 children have been examined with 270 bronchoscopic examinations. Some of the children were bronchoscoped as many as nine times, and the most important findings may be seen in table 1. The table is so arranged that the most important finding for each child is placed in the first column, while the remaining findings are placed in the indented column, thus recording all of the pathologic findings in the bronchial tree for these 90 children. If, for example, a lymph node perforation into the bronchial tree is found, it is listed in the first column. If at the same time there is substantial lymph node compression of the bronchial tree with purulent secretion, this is registered in the indented column, and so on.

TABLE 1

Segmental Atelectasis: Bronchoscopic Findings

Bronchoscopic Findings	Groups		
	I	II	Total
Stenosis			
Lymph node compression	37	24	61 (67.8%)
Granulations	5	21	26
Scar tissue	1	4	5
Mucosal changes			
Widespread	10	56	66
Limited	3	13	16
Broadening of carina		36	36
Purulent secretion from one or more bronchial ostia	4	48	52
Lymph node perforations	25		25 (27.8%)
Threatened perforations	4		4
Negative bronchoscopic findings	1		1
Number of children	90		
Bronchoscopic examinations	270		

At the bronchoscopic examinations it was found that 61 of the 90 children had visible bronchial stenosis as a result of lymph node compression, i.e., 67.8 per cent. Bronchial stenosis as a result of granulations was present in 26 children, and bronchial perforations were also present in 13 of these. In 5 cases bronchial stenosis because of scar tissue was found. Widespread mucosal changes were found in 66 patients, in 10 of whom this was the most important finding. More limited mucosal changes were found in 16 cases, broadening of the carina in 36 cases, and purulent secretion from bronchial openings in 52 cases.

Lymph node perforations to the bronchial tree were found in 25 of the 90 children, that is, in 27.8 per cent. In an additional 4 cases, signs of threatened perforation were present, but no perforation took place during the observation period. There was only one patient with completely negative bronchoscopic findings.

The lung segments that were found atelectatic in the 90 children may be seen in figure 1. In all, 174 segments were involved, the right lung predominating with 111 segments as compared with 63 segments on the left side. There was a predominance of involvement of the anterior segments of the right lung, both the anterior segments of the upper lobe and the middle lobe. This is probably explicable on the basis of special anatomic relationship to the bronchial tree on the right side.

In 32 of the 90 children with segmental atelectasis, tubercle bacilli were demonstrated by direct microscopy of the sputum in 2, by cultivating the sputum in 5, and by cultivation of laryngeal swabs or gastric lavages in an additional 25. Drug-susceptibility tests showed that all of these strains of tubercle bacilli were susceptible to streptomycin, isoniazid, and PAS. In 28 children, circular shapes which were interpreted as cavities or suspected cavities were demonstrated in the chest films (usually with planigrams); in 18 of these children, tubercle bacilli were demonstrated.

The localization of the lymph node perforations in the bronchial tree may be seen in figure 2. As 3 of the children had two perforations each, there were twenty-eight lymph node perforations noted in the 25 children. This does

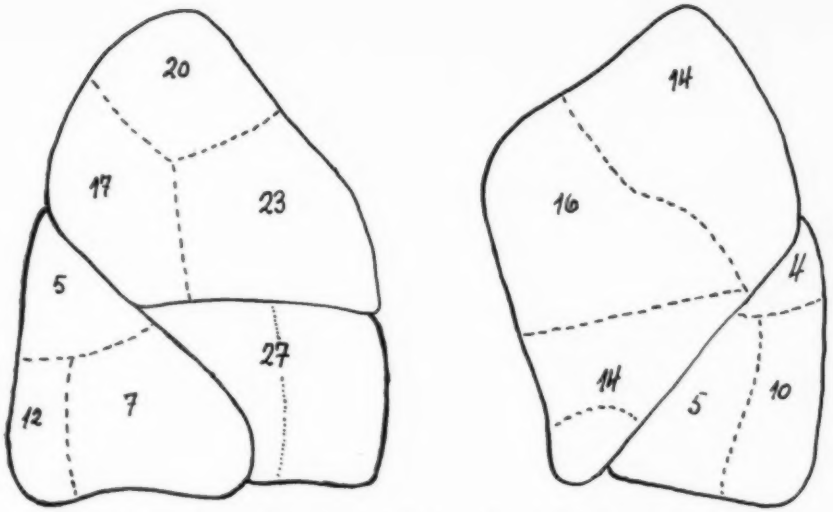

FIG. 1. A record of the lung segments that were found atelectatic in 90 children.

not include the cases of granulation tissue in the bronchial tree in which there was no direct evidence of perforation. As may be seen, the majority of the perforations were found on the right side: twenty-one perforations in contrast to seven perforations on the left side. Thus, there were three times as many perforations on the right side as on the left. This can probably be explained by the anatomic relationship to the bronchial tree on the right side. The pulmonary artery encircles the bronchial tree in a closer way on the right than on the left side. Moreover, as Rouviére (15) has shown, the lymph nodes on the right side take part in the draining of the lymph from the left lung, at any rate from its basal and middle parts. This, however, should apply especially to the upper interlobar and the paratracheal lymph nodes, so this explanation does not suffice to elucidate the great accumulation of lymph node perforations in the stem and the middle lobe bronchi.

In table 2 are shown the appearance of the lymph node perforations in relation to the primary infection, which for some of the children can be determined fairly accurately and for the others from the time they were first found to be tuberculin positive. One of the 25 children had lymph node perforation at two different periods, respectively one month and eleven months after the primary infection. It may be seen in table 2 that 11 lymph node perforations appeared in the first half year after the primary infection, with a slight increase four to six months after primary infection. In the second half of the year, 9 cases appeared, fairly equally distributed in the different months of this period. Later, the frequency of the perforations decreased rapidly, but lymph node perforations were noted as late as thirty-three months after primary infection. These cases of lymph node perforation need relatively prolonged treatment, and it has been the writer's practice to continue the treatment as long as inflammatory changes can be found in the bronchial tree.

The duration of the treatment in the 25 cases of lymph node perforations is shown in table 3. Of 3 patients who were discharged from the hospital before one and a half years of treatment, one was discharged against medical advice.

The age distribution and the distribution between the two sexes may be seen in table 4. The greatest number of cases of lymph node perforations were found in the small children and in the younger school-age group. Lymph node perforations were found in 16 of 47 boys and in 9 of 43 girls. The difference corresponds approximately to twice the standard deviation

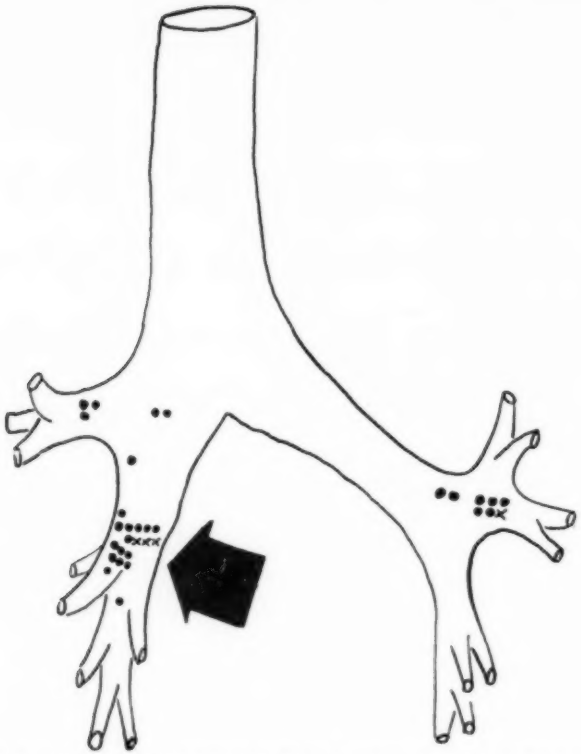

FIG. 2. Localization of the lymph node perforations in the bronchial tree
●—28 lymph node perforations in 25 children
×—threatened perforations

TABLE 2

TIME OF APPEARANCE OF LYMPH NODE PERFORATION IN RELATION TO PRIMARY INFECTION

Number of Months	Lymph Node Perforation	
0–2	3	
2–4	3	11
4–6	5	
6–8	3	
8–10	3	9
10–12	3	
12–18	4	
18–24	1	
24–36	1	

of the difference and is therefore at the limit of being statistically significant (16).

The symptoms produced by the lymph node perforation in the 25 children may be seen in table 5. The lack of symptoms in children with these perforations is remarkable. Seven of the 25 children had coughs, but none had that paroxysmal irritative cough which is found in adults. In only one case was a diagnosis of "whooping-cough" made just before admission to the hospital. The "whooping-cough" was judged to represent a symptom of a lymph node perforation. Coughing was negligible in the remaining 6 children and was expressed as "slight cough" in the nurses' reports despite the fact that the nurses were aware of the threat of perforation.

Only 3 of the 25 children had abnormal bronchial sounds, and the subsequent bronchoscopic examination of one of these verified the diagnosis of lymph node perforation. Perforation had already been detected in the other 2 children when the bronchial sounds became evident. Only one child coughed up caseous matter

TABLE 3
LYMPH NODE PERFORATION: DURATION OF TREATMENT

Number of Patients	Duration of Treatment
10	Still under treatment
2	For less than 12 months
1	12–18 months
8	18–24 months
3	24–30 months
1	More than 30 months

TABLE 4
LYMPH NODE PERFORATION: AGE DISTRIBUTION

Age	Total	Boys	Girls
years			
0–2	5	4	1
2–4	6	4	2
4–6	6	3	3
6–8	4	2	2
8–10	2	2	0
10–12	1	0	1
12–14	1	1	0
Total perforations	25	16	9
Material observed	90	47	43

from the lymph node, but children frequently swallow what they cough up. Eight children had fever, and among these are included 3 extremely doubtful cases, in which there was scarcely any connection between a slight rise in temperature and the lymph node perforation. The erythrocyte sedimentation reaction was abnormal in 10 cases; in 3 cases the sedimentation rates showed no change; and in 12 cases the rates were decreasing at the time of lymph node perforation. In this symptom deficiency, the weights of these children were followed accurately. Eight children lost weight, 12 children showed no weight increase, and the remaining 5 showed very slow weight increase.

It has often been possible to trace, coincident with the lymph node perforation, a simultaneous clearing of the atelectatic zone in the lung or obvious regressive changes on the routine chest film or on the planigrams. Not infrequently this sudden change in a regressive direction led to the correct diagnosis, namely, lymph node perforation. This was also verified at the bronchoscopic examinations which followed. In this observation of 25 lymph node perforations, obvious regressive changes on the roentgenogram have been noted in 20 cases. In one case the regression was gradual, in another case the regression was questionable, and in 3 cases the atelectasis continued unchanged. (Of these last 3 cases, one child was operated on at an early stage, another child had lymph node perforation on one side and in the atelectatic zone on the opposite side of the bronchial tree, and the third child had perforation and atelectasis on the same side but in different parts of the bronchial tree).

The duration of the antituberculous chemotherapy before lymph node perforation appeared may be seen in table 6. Only 2 children had been untreated at the time of perforation, whereas all of the other 23 children had received some antimicrobial therapy. The data in the table reveal that even after twelve to fourteen months of chemotherapy a lymph node perforation may occur.

In the entire series of 90 children with segmental atelectasis, there were only 4 who did not receive chemotherapy. The majority received triple-drug therapy, i.e., streptomycin, isoniazid, and PAS. As was customary, four laryngeal

TABLE 5
LYMPH NODE PERFORATION: SYMPTOMS IN 25 CASES

Coughing	7
Abnormal bronchial sounds	3
Coughing up of lymph node content	1
Fever (including 3 doubtful cases)	8
Erythrocyte sedimentation rate	
Increased	10
Unaltered	3
Decreased	12
Loss of weight	8
No weight increase	12
Slow weight increase	5

TABLE 6
DURATION OF CHEMOTHERAPY BEFORE LYMPH NODE PERFORATION

Number of Patients	Chemotherapy Before Perforation
	months
2	Untreated at perforation
3	1–2
6	2–4
6	4–6
1	6–8
2	8–10
3	10–12
2	12–14

swabs were cultivated on all of the discharged patients, and all except one child were free from bacilli at this examination. The child with bacilli had persisting cavities in the lingular segment on the left side which were later resected. The tubercle bacilli were only moderately susceptible to streptomycin (growth at 10 γ per ml., but no growth at 50 γ per ml.); relatively resistant to isoniazid (growth at 3 γ per ml., questionable growth at 10 γ per ml.); and relatively resistant to PAS (growth at 10 γ per ml., no growth at 50 γ per ml.); and produced no tuberculosis in guinea pigs.

In table 7 may be seen the findings at routine bronchographic examinations of 33 children at the end of the therapy period. From the table it may be seen that 27 of these 33 bronchograms revealed abnormalities. Bronchiectasis was found in 12 cases, bronchostenosis in 16. In 7 cases the bronchi were dislocated as a result of severe shrinking of the lung segment, and in 7 cases bronchial branches were compressed together as a result of intense fibrosis of the corresponding segments. Finally, in 2 cases minor pathologic changes were found in the bronchial wall itself.

This great frequency of pathologic findings in the bronchial tree after segmental affections is not surprising, as other investigators have found a very large incidence of bronchiectasis, for example, in cases of the so-called epituberculosis. In the same way, Roberts and Blair (17) have found 46 cases of bronchiectasis among 77 cases of segmental atelectasis. Jones and her co-workers (18–20) found bronchiectasis in 58 per cent of patients in whom the atelectasis had lasted for more than twelve months, and in 70 per cent of patients three and a half years after all signs of epituberculosis had disappeared. Jeune and his co-workers (21–25) report bronchiectasis in 72 to 77 per cent, and Rogstad (26, 27) found bronchiectasis in 12 of 26 cases at planigraphic examination. The findings in the present series are therefore in agreement with the findings of other investigators.

TABLE 7

SEGMENTAL ATELECTASES: BRONCHOGRAPHIC FINDINGS

Negative findings	6
Pathologic findings	27
Bronchiectasis	12
Bronchial stenosis	16
Dislocation of bronchial branches	7
Fibrosis (compact bronchial branches)	7
Bronchial wall changes	2

It should be true, as has also been emphasized, that not all of these instances of bronchiectasis will give symptoms later on. Bronchiectasis in the upper lobe seems to be sufficiently well drained so that it should be an unlikely cause of symptoms in the future. On the other hand, bronchiectasis in all branches of the lower lobe, in the middle lobe on the right side, and in the lingular segment on the left side is more liable to be infectious. The insufficient drainage from these lower segments could easily give the well-known symptoms of this disease. When extensive bronchiectasis is found in these parts of the lung, resection is recommended, and examination of the resection specimens has confirmed the correctness of this viewpoint.

A total of 31 segments has been resected in 15 children with segmental atelectasis. Without going into details concerning the pathologic findings, it can be stated that, in addition to bronchiectasis, atelectasis, and lung fibrosis, there were also signs of a progressive tuberculosis in the lung segments in as many as 12 of the 15 cases; and in 3 of these, persistent cavities were noted. There were only 3 cases in which there was no evidence of tuberculosis in the resected specimens, but in which extensive fibrosis of the lung and also ectasis of the bronchi were present. It is quite amazing to see the extent this shrinking process can reach.

The significance of the source of a primary infection in relation to its course has been emphasized in the past. It has been asserted that an intimate contact with a tuberculous mother is more apt to be associated with a fatal outcome of the primary infection.

The different sources of the infections of the present series are reported in table 8. In 20 cases a tuberculous father was the source of infection; and in 18 cases, a tuberculous mother. In an additional 18 cases the source was an uncle or an aunt; and in 9 cases there were others in the family with tuberculosis who were pointed out as sources of infection. A tuberculous neighbor was the apparent infection source in another 11 cases, and in 14 cases it was not possible to find any known source of infection.

Two cases of tuberculous meningitis developed among the 90 children with segmental atelectasis.

TABLE 8

SEGMENTAL ATELECTASIS: SOURCE OF INFECTION

Number of Cases	Contacts	Lymph Node Perforations			
		Total	Boys	Girls	Dead
20	Tuberculous father	9	7	2	1
18	Tuberculous mother	3	1	2	
18	Tuberculous uncle or aunt	3	1	2	
9	Other tuberculous family members	2	1	1	
11	Tuberculous neighbor	6	5	1	
14	Unknown infection source	2	1	1	

In the one case, the tuberculous disease started with meningitis; in the other case, the tuberculous meningitis developed two months after the patient returned home from a sixteen-month sojourn at the hospital for his primary tuberculosis. This patient, a 3½-year-old-boy, had also lymph node perforation in the bronchial tree during the second hospital sojourn, thirty-three months after the primary infection. A third case of meningitis was observed, but this case does not belong in the same category, as there was no segmental atelectasis. All 3 cases of tuberculous meningitis were brought under control with chemotherapy, and the 3 children are alive and well twenty, thirty-five, and forty-three months after the appearance of the tuberculous meningitis.

Two patients with keratitis phlyctenularis and one with tuberculous otitis media have been observed and represent the only extrapulmonary tuberculosis noted. In all children in the series three specimens of urine were cultivated before chemotherapy was started, but no evidence of infection was found in any case. It must be added, however, that the observation period for a number of the children concerned is obviously too short to record all later manifestations of tuberculosis in other organs apart from the lung. It is to be hoped, however, that the chemotherapy will have resulted in the healing of any disseminated lesions that might have occurred. The course of segmental atelectasis seems to be essentially unchanged from the pre-chemotherapy era. The lymph node perforations seem to appear despite the therapy, and the residual conditions in the lungs after a segmental atelectasis, with or without lymph node perforation, seem just as serious now as in the pre-chemotherapeutic period. It appears, therefore, that there is good reason to find other methods of therapy of segmental atelectasis than those in use today. Whether these new methods will consist of roentgen therapy of the hilar and mediastinal lymph nodes, in relatively early surgical lymph node excision, or in corticosteroid therapy remains to be seen.

SUMMARY

The great frequency of serious bronchoscopic findings in a series of 90 children with segmental atelectasis following primary tuberculosis is recorded. Bronchial stenosis relating to lymph node impression was present in 67.8 per cent; and lymph node perforation of the bronchial tree, in 27.8 per cent. In only one child were the findings completely negative at bronchoscopic examination. Both the atelectasis and the complications, such as lymph node perforation, were more frequently found on the right side.

Lymph node perforation was found more often in children younger than the school-age group, and more frequently in boys than in girls. Lymph node perforations in children very often appeared without symptoms (very few children had any symptoms at all), but coincident with these perforations a simultaneous clearing of the atelectatic zones was observed in the lungs of the children. This symptom not infrequently led to the correct diagnosis—the lymph node perforation—being verified at the next bronchoscopic examination. The perforations appeared in spite of antituberculous chemotherapy, and even twelve to fourteen months of chemotherapy did not prevent a lymph node perforation.

Bronchographic examination of 33 of the children showed abnormal bronchograms in 27. Tuberculosis was found in 12 specimens resected from 15 children; in addition, bronchiectasis, atelectasis, and lung fibrosis were found.

In contrast to other reports, it was found that an intimate infectious contact with a tuberculous mother does not have a more serious course than contacts with other sources of infection.

SUMARIO

La Atelectasia Segemental en los Niños con Tuberculosis Primaria

Se hace notar la gran frecuencia alcanzada por los hallazgos broncoscópicos graves en una serie de

90 niños que tuvieron atelectasia segmental a continuación de una tuberculosis primaria. En 67.8 por ciento, había estenosis bronquial relacionada con la impresión de los ganglios linfáticos y en 27.8 por ciento perforación de los ganglios al árbol bronquial. Sólo en un niño resultaron los hallazgos absolutamente negativos en el examen broncoscópico. Tanto la atelectasia como las complicaciones, tales como la perforación de los ganglios linfáticos, fueron más frecuentes en el lado derecho.

La perforación de los ganglios linfáticos se descubrió más a menudo en los niños que no habían llegado a la edad del grupo escolar, y más frecuentemente en los varones que en las mujeres. Las perforaciones de los ganglios linfáticos en los niños aparecieron muy a menudo sin síntomas (muy pocos niños mostraron el menor síntoma), pero coincidiendo con estas perforaciones, se observó un despejo de las zonas atelectáticas en los pulmones de los niños. Este síntoma condujo bastantes veces al diagnóstico acertado—perforación de los ganglios linfáticos—que se formuló en el siguiente examen broncoscópico. Las perforaciones aparecieron a pesar de la quimioterapia antituberculosa, sin que las impidieran ni de doce a catorce meses de quimioterapia.

Los exámenes broncográficos de 33 de los niños rindieron un broncograma patológico en 27 de ellos; en los ejemplares resecados, después de la resección segmental en 15 niños, además de bronquiectasias, atelectasias, fibrosis pulmonar, etc., se descubrió tuberculosis en 12.

En contraposición a lo expuesto por otros autores, se observó que el contacto íntimo con una madre tuberculosa no producía una evolución más grave que los contactos con otros focos infecciosos.

Se discute el tratamiento actual de las atelectasias segmentales, ofreciéndose la opinión de que hay las mejores razones para renovar los esfuerzos encaminados a descubrir nuevos métodos terapéuticos para dichos casos.

Resume

Atélectasie segmentaire chez les enfants atteints de tuberculose primaire

L'auteur signale la grande fréquence d'importants signes bronchoscopiques enregistrés parmi une série de 90 enfants atteints d'atélectasie segmentaire à la suite d'une primo-infection tuberculeuse. La sténose bronchique, en relation avec la pression exercée par un ganglion lymphatique, était présente dans 67,8% des cas, et la perforation du ganglion lymphatique dans l'arbre bronchique dans 27,8% des cas. Il n'y a que chez un seul enfant que l'examen bronchoscopique a été complètement négatif. L'atélectasie et les complications, telle la perforation du ganglion lymphatique, étaient plus fréquemment découvertes du côté droit.

La perforation ganglionnaire était décelée plus souvent chez les enfants plus jeunes que dans le groupe scolaire et plus fréquemment chez les garçons que chez les filles. La perforation des ganglions lymphatiques chez l'enfant est souvent asymptomatique (très peu d'enfants ont présenté des symptômes quels qu'ils soient); cependant, en même temps que se produisaient ces perforations, l'éclaircissement des zones d'atélectasie était observé. Ce signe a souvent conduit au diagnostic exact—la perforation du ganglion lymphatique—qui était vérifiable lors d'une bronchoscopie ultérieure. Les perforations se sont produites en dépit de la chimiothérapie antituberculeuse, et douze à quatorze mois de chimiothérapie n'ont pas empêché une perforation du ganglion lymphatique.

Les examens bronchographiques pratiqués chez 33 des enfants ont révélé un bronchogramme pathologique chez 27 d'entre eux: dans les pièces d'exérèse après résection segmentaire chez 15 enfants, on a découvert la tuberculose dans 12 cas, plus des bronchiectasies, des atélectasies, des scléroses pulmonaires, etc.

En contraste avec les autres rapports, il a été observé que le contact intime avec l'infection, avec une mère tuberculeuse, par exemple, n'entraîne pas une évolution plus grave que le contact avec d'autres sources d'infection tuberculeuse. Le traitement actuel des atélectasies segmentaires est discuté, et les auteurs sont d'avis qu'il y a lieu de renouveler les efforts pour découvrir des méthodes thérapeutiques nouvelles s'appliquant à de tels cas.

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PLEURAL FLUID AMYLASE IN PANCREATITIS AND OTHER DISEASES[1, 2]

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Introduction

Acute pancreatitis may be accompanied by pleural effusion. Schieppati and Vernengo (1) in 1946 reported 5 patients with acute pancreatitis and pleural effusion. The effusions in 4 of these 5 patients were on the left side. Lipp and Aaron (2) found that 22 of 31 patients with pancreatitis (71 per cent) had chest involvement. Twelve had pleural effusions and 10 had atelectasis. The pleural effusions were usually on the left. Coffey (3) reported 6 instances of pleural effusion in 135 patients with pancreatitis. In all 6, the effusion was on the left. Meyers and associates (4) recorded chest abnormalities in 6 per cent of 126 patients but did not specify how many had pleural effusions. Individual cases have been reported by Werner (5), de Takats and Mackenzie (6), Glenn and Baylin (7), Gambill and coworkers (8), Smith (9), Kalser and associates (10), Juniper (11), and Byrd and Couch (12). Of 31 cases of pancreatitis in which information was given, 21 effusions were on the left only, 3 on the right, 7 were bilateral.

The amylase content of the pleural fluid was measured in the acute phase in only a few instances. These are listed in table 1. Only one study was found in which the amylase content of pleural fluid was measured in other diseases. Werner determined the amylase content of pleural fluid in 7 patients without pancreatitis. The pleural fluid amylase was invariably low, and in most instances it was lower than the serum value.

It is the purpose of this paper to report on additional patients with pleural effusion due to pancreatitis, to report the pleural fluid amylase in other diseases, and to emphasize the potential significance of determinations of the pleural fluid amylase.

Observations

Case Reports

Following are 4 cases of pancreatitis with pleural effusion and serial pleural amylase determinations. In one of these cases, the original diagnosis was first suspected after thoracentesis and amylase determination on the pleural fluid.

Case 1. A 50-year-old Negro male chronic alcoholic was admitted to the Veterans Hospital on October 14, 1955, with a two-week history of abdominal pain, distention, and anorexia. One week prior to admission, he had developed a productive cough and pain in the left chest. The patient was a diabetic. His diabetes was controlled with isophane insulin until shortly before admission, when he became comatose.

The physical examination revealed a chronically and acutely ill unconscious male. The blood pressure was 140 systolic and 86 diastolic in mm. of mercury; the pulse rate was 110; the respiratory rate, 20. The examination of the lungs disclosed dullness in the left chest. Laboratory data included normal hemoglobin, leukocyte count, serum bilirubin, blood urea nitrogen, and alkaline phosphatase. The serum amylase was 1,070 units. The chest roentgenogram showed a left pleural effusion (figure 1). A gastrointestinal examination after a barium meal displayed forward displacement of the stomach and widening of the duodenal sweep.

On admission to the hospital, a left thoracentesis was done, and 2,000 ml. of serosanguineous fluid was removed with an amylase concentration of 53,000 units per ml. The serum amylase was 1,070 units per ml. The pleural fluid contained 1.5 gm. of protein per 100 ml. with a specific gravity of 1.018. On October 20, 1955, another thoracentesis yielded fluid with an amylase of 22,800 units per ml. On October 26, 1955, the pleural fluid amylase was 3,200 units per ml. The amylase determinations are tabulated in table 2.

The patient improved after treatment for diabetic coma. On November 14, 1955, the patient had a distal pancreatectomy with a gastro-pancreatic anastomosis. On January 23, 1956, a dilation of the pseudocyst gastrostomy and a posterior gastrojejunostomy were done. The pancreatic cyst fluid had an amylase of 26,600 units per ml. The patient became asymptomatic and was discharged from the hospital.

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[2] Presented before the Medical Session, as part of *Section 3B*, at the annual meeting of the American Trudeau Society, Philadelphia, Pennsylvania, May 20, 1958.

Case 2. A 34-year-old Negro female, who had been a known alcoholic for eight years, was admitted to the University Hospital on October 9, 1955, with severe abdominal pain and hematemesis. The patient was pregnant at the time of admission.

The physical examination revealed an acutely ill gravid female with abdominal pain and tenderness in the right upper quadrant. The blood pressure was 100 systolic and 70 diastolic in mm. of mercury, the pulse rate 104, and the temperature 103°F.

TABLE 1

MEASURE OF AMYLASE CONTENT OF PLEURAL FLUID IN ACUTE PHASE FROM REPORTS IN LITERATURE

	Pleural Fluid Amylase	Serum Amylase
	units/ml.	*units/ml.*
Schieppati and Vernengo (1)		
Case 1	0	
Case 2	1,024	
Case 5	0	100
Gambill *et al.* (8)	50	< 50 (had been 2,666)
Kalser *et al.* (10)	1,723	1,020
Juniper (11)	800	800
Werner (5)	2,048	64

The laboratory work revealed hemoglobin of 13 gm. per 100 ml., a hematocrit of 41, and a leukocyte count of 18,000 per mm.^{3} with 92 per cent polymorphonuclear cells. A urinalysis was negative for sugar, but acetone and albumin tests were 2-plus. A fasting blood sugar was 55 mg. per 100 ml. The total protein was 5.8 gm. with 2.6 gm. of albumin and 3.2 gm. of globulin.

On October 19, 1955, the patient developed shock. A roentgenogram of the chest revealed a left pleural effusion. A thoracentesis was performed; and 200 ml. of serosanguineous fluid were removed. The amylase determination of this fluid was 1,136 units per ml. and the serum amylase was 243 units per ml. The amylase determinations are listed in table 3.

The patient responded only intermittently to measures to correct the shock. She delivered a dead fetus and died on October 26, 1955. A postmortem examination confirmed the presence of acute pancreatitis with fat necrosis involving the omentum. A benign gastric ulcer was also found.

Case 3. A 31-year-old white male, who was a known alcoholic for ten years, was admitted to the Veterans Hospital on November 29, 1955, with severe abdominal pain of two weeks' duration. He had had intermittent episodes of jaundice for about two years.

Physical examination revealed an acutely ill, jaundiced male. The blood pressure was 150 mm. systolic and 80 diastolic in mm. of mercury, the

FIG. 1. Film illustrating a left pleural effusion.

TABLE 2

AMYLASE DETERMINATIONS IN CASE 1

	Day	Amylase		Cyst Fluid
		Serum	Pleural Fluid	
		units/ml.	*units/ml.*	*units/ml.*
Oct. 15, 1955	1	1,070	53,000	
	2	640		
	3	533		
	4		22,800	
	5	775		
	6		3,200	
	7	1,150		
	8	1,450		
Jan. 6, 1956		69		
Jan. 20, 1956		200		
Jan. 23, 1956				26,600

TABLE 3

AMYLASE DETERMINATIONS IN CASE 2

Day	Amylase	
	Serum	Pleural
	units/ml.	*units/ml.*
1	243	1,136
2	141	
3		
4	164	

pulse rate was 120, and the temperature 103°F. The abdomen was distended with ascites and the liver was palpable 4 cm. below the costal margin. Percussion revealed dullness of the left chest. There was 2-plus pitting edema of the ankles.

The urinalysis was normal; leukocyte count was 6,050 per mm.^{3} with 70 per cent neutrophils; hemoglobin was 12.5 gm. per 100 ml. A serologic test for syphilis was negative. The total serum bilirubin was 9.2 mg.; the total protein was 5.4 gm. per 100 ml. with 2.4 gm. of albumin; a fasting blood sugar was 85 mg. per 100 ml.; the serum amylase was 725 units per ml. The chest roentgenogram disclosed a left pleural effusion (figure 2).

After admission to the hospital, a left thoracentesis yielded 300 ml. of serous fluid with an amylase concentration of 800 units per ml. The protein content was 3.8 gm. per 100 ml. The amylase results are given in table 4. A paracentesis was done and the amylase content of the ascitic fluid was 114 units per ml. The patient responded to medical management and became asymptomatic. A liver biopsy revealed Laennec's cirrhosis. He was discharged after disappearance of his jaundice.

Case 4. A 36-year-old white male, who was a known alcoholic for eight years, was admitted to the Veterans Hospital on August 2, 1957, with abdominal pain, nausea, and vomiting. A diagnosis of pancreatitis was made in October, 1954, following a similar episode.

The physical examination revealed an acutely ill man. The abdomen was rigid. No organs or masses were palpable. The blood pressure was 160 systolic and 90 diastolic in mm. of mercury; the pulse rate, 110; and the temperature, 99°F. The laboratory work included hemoglobin of 10 gm. per 100 ml., a hematocrit of 36 per cent, and a leukocyte count of 19,000 per mm.^{3} with 80 per cent neutrophils. The urinalysis was negative except for a 3-plus sugar. The fasting blood sugar was 182 mg. per 100 ml. The serum amylase was 1,600 units per ml. The total protein was 6.2 gm. per 100 ml. with 2.4 gm. of albumin.

Continuous gastric suction was begun. On August 9, 1957, ascites developed and, on August 15, 1957, a roentgenogram of the chest revealed a right pleural effusion (figure 3). A thoracentesis was performed, and 560 ml. of serous fluid were removed. The amylase concentration of the pleural fluid was 3,200 units per ml. On August 17, 1957, a pleural fluid amylase was 1,140 units per ml. and on August 19 the pleural fluid amylase was 350 units per ml. These results are listed in table 5. A gastrointestinal series revealed an obstruction of the duodenum by an extrinsic mass. On August 31, 1957, an exploratory laparotomy was performed and a large pancreatic cyst was found displacing the duodenum medially. This was drained with a cystoduodenostomy. The cyst fluid amylase was 30,000 units per ml. The patient's postoperative course was uneventful and he was discharged from the hospital.

Amylase content in pancreatitis: The pleural fluid amylase determinations in the 4 cases of pancreatitis complicated by pleural effusion are summarized in table 6. A modification of the method of Comfort and Osterberg (13) was used. In all 4 cases, the pleural fluid amylase was higher than the serum amylase and returned to normal more slowly than the serum amylase in the 2 patients in whom serial determinations were performed. The mean pleural fluid amylase was 14,334 units per ml. and the mean for the serum was 367 units per ml.

Amylase content in other diseases: The pleural fluid amylase was determined in 12 patients with diseases other than pancreatitis. The results are given in table 7. In all instances in which the determinations were performed on the same day, the pleural fluid amylase was less than the serum amylase. The mean pleural fluid amylase was 81,

FIG. 2. Film illustrating a left pleural effusion.

FIG. 3. Film illustrating a right pleural effusion.

and the serum amylase, 158. This difference is statistically significant ($t = 2.86$, $P < 0.01$).

Roentgenographic findings in pancreatitis: Sixty-three cases of pancreatitis have been observed at the University and Veterans Administration hospitals in the past ten years; 30 per cent of the patients had roentgenographic chest involvement. Fourteen showed atelectasis and 5 had pleural

effusions. An additional 4 patients had significant pleural effusions found at post-mortem examination. Two of these had atelectasis reported on the chest roentgenogram and 2 had normal chest films. In these instances the roentgenographic examinations were performed several days before death. The total number of patients with pleural effusions was therefore 9, or 15 per cent.

TABLE 4

AMYLASE DETERMINATIONS IN CASE 3

Day	Amylase		Peritoneal Fluid
	Serum	Fleural Fluid	
	units/ml.	*units/ml.*	*units/ml.*
1	725	800	
3	266		
8	267		
12			114
13	107		
19	266		

TABLE 5

AMYLASE DETERMINATIONS IN CASE 4

Day	Amylase		Cyst Fluid
	Serum	Pleural Fluid	
	units/ml.	*units/ml.*	*units/ml.*
1	1,600		
4	800		
6	400		
7		3,200	
11	320		
12		1,140	
15	266		
16		350	
22			22,000
27	100		

TABLE 6

SUMMARY OF AMYLASE DETERMINATIONS IN PRESENT CASES

	Amylase	
	Pleural	Serum
	units/ml.	*units/ml.*
Case 1	53,000	1,070
Case 2	1,136	243
Case 3	800	725
Case 4	3,200	400
Mean	14,334	367

TABLE 7

AMYLASE DETERMINATIONS IN PATIENTS WITH DISEASE OTHER THAN PANCREATITIS

Number	Disease	Amylase	
		Pleural	Serum
		units/ml.	*units/ml*
1	Heart failure	55	160*
2	Heart failure	114	266
3	Heart failure	51	80
4	Tuberculosis	<20	—
5	Tuberculosis	88	288
6	Tuberculosis	40	52
7	Carcinoma of lung	133	80*
8	Hodgkin's disease	80	123
9	Cirrhosis	123	200
10	Empyema	50	123
11	Postpneumonic effusion	76	80
12	Subdiaphragmatic abscess	145	288
	Mean	81	158
	Standard deviation	40	89

* Not determined the same day

DISCUSSION

Pleural effusion and other pulmonary findings frequently complicate pancreatitis (2, 3). It is almost certain that the amylase found within the pleural cavity has the pancreas as its original source. However, it is not known how the pancreatic enzymes reach the pleural cavity and other parts of the body.

Several theories have been postulated for the relationship of pleural effusion to pancreatitis. There is a rich lymphatic connection between the abdomen and thorax through the diaphragm. These connections serve as auxiliary channels and probably are used only during times of obstruction of the normal lymph drainage. This certainly would be present in pancreatitis with its inflammatory reaction (10). Another possible explanation is that of direct contact and fat necrosis (3, 7). Anatomically, the tail of the pancreas comes in direct contact with the diaphragm. With the acute inflammation of pancreatitis, direct contact with the pancreatic secretion may give the needed chemical irritation to cause the pleural effusion, and with retrograde lymph drainage to maintain the elevated pleural amylase. It is interesting that one of the patients in the present report had a right pleural effusion. His disease was complicated by a large pseudocyst of the head of the pancreas extending into the right upper quadrant. Direct inflammation of the right diaphragm may account for this. The

remaining 3 patients had left pleural effusions, and only one of these had a pseudocyst of the pancreas.

It is evident that, regardless of the pathogenesis of the effusions seen in pancreatitis, an appreciable number of patients do have abnormal chest roentgenographic findings. This indicates that roentgenographic examination of the chest will be of aid in the diagnosis. Furthermore, when fluid is suspected, a thoracentesis with examination of the fluid for its amylase content may confirm the diagnosis of pancreatitis.

Summary

Four patients with pleural effusion due to pancreatitis are reported. The amylase content was invariably elevated, was higher than the serum concentration, and declined more slowly. The pleural fluid amylase was determined in other patients with pleural effusions due to diversified causes. The pleural fluid amylase was invariably lower than the serum amylase.

Abnormal chest roentgenograms were discovered in 30 per cent of 63 patients with pancreatitis. Atelectasis was the most common abnormality, but effusions were present in 15 per cent.

Sumario

La Amilasa del Liquido Pleural en la Pancreatitis y Otras Enfermedades

Preséntanse los casos de 4 enfermos que tenían derrame pleural debido a pancreatitis. El contenido de amilasa estaba invariablemente elevado, era superior a la concentración sérica y descendió con mayor lentitud. También se determinó la amilasa del liquido pleural en otros enfermos que tenían derrames pleurales debidos a diversas causas. La amilasa del liquido pleural resultó invariablemente más baja que la amilasa sérica.

En 30 por ciento de 63 enfermos de pancreatitis se observaron radiografías torácicas anómalas. La atelectasia era la anomalía más común, pero había derrames en 15 por ciento.

Resume

Amylase du liquide pleural dans la pancréatite et autres affections

L'observation de quatre malades atteints d'épanchement pleural dû à une pancréatite est rapportée. La teneur de l'épanchement en amylase était invariablement élevée; elle était plus élevée que dans le sérum et elle diminuait plus lentement. L'amylase du liquide pleural a été déterminée chez d'autres malades atteints d'épanchement pleural dû à diverses causes. La concentration de l'amylase dans le liquide pleural était toujours plus faible que celle du sérum.

Des radiogrammes anormaux du thorax ont été découverts chez 30% des 63 malades atteints de pancréatite. L'atélectasie pulmonaire était l'anomale la plus commune; l'épanchement était présent dans 15% des cas.

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RELAPSE RATE IN A TWO- TO ELEVEN-YEAR FOLLOW-UP STUDY OF PATIENTS WITH PULMONARY TUBERCULOSIS TREATED WITH AND WITHOUT ANTIMICROBIALS AND DISCHARGED FROM 1946 THROUGH 1955[1]

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Introduction

The reduction in mortality from tuberculosis is obvious evidence of the effectiveness of the chemotherapy of tuberculosis, and world-wide reports make it possible to form an estimate of this effectiveness. A less spectacular but no less important indication of this effectiveness may be obtained by comparing the relapse incidence following treatment without drugs and treatment with drugs. But while there have been numerous reports on relapses in pulmonary tuberculosis following treatment in the pre-chemotherapy era (1–8), there are fewer reports in the literature of relapses following treatment with drugs (9–13).

All reports of relapses in the pre-chemotherapy era agree that the larger proportion of relapses, 50 to 70 per cent, takes place in the first two or three years following discharge. Studies of relapses in this institution (Fort Qu'Appelle Sanatorium) have been published twice previously, by Ferguson in 1926 (1) and by Wherrett in 1935 (2), and it seemed timely to make a comparison of relapses among patients treated here in the years just before chemotherapy and in the years of chemotherapy. A study of relapses among dischargees from 1946 through 1955 would furnish five-year material of patients nearly all treated without drugs, and successive five-year material of patients nearly all treated with drugs. At the end of 1957 there would be a follow-up period of these cases from two to eleven years.

Material and Method

Patients with pulmonary tuberculosis, treated and discharged alive from the Fort Qu'Appelle Sanatorium between 1946 and 1955, form the material for this study. The following groups were not included in the study: those with extrapulmonary manifestations, those with pleurisy only, obvious primary tuberculosis, and Indian patients.[2]

There were 1,225 cases available, and from these the following were discarded: those who had had resectional surgery, those who had had no follow-up reviews after discharge, and those who had had what, for the purpose of this study, is considered to be "inadequate treatment." A patient is considered to have had inadequate treatment if his treatment in the sanatorium lasted for less than six months. Since the main purpose of this study is a comparison of relapse incidence in patients treated without antimicrobials and patients treated with antimicrobials, and since it is generally accepted that prolonged treatment with antimicrobials for at least six, eight, or twelve months is necessary to stabilize a lesion, a period of six months has arbitrarily been set as a minimal period of treatment required. In order to make the two series of cases as nearly comparable as possible, the same minimal period of treatment therefore has been set for patients treated without antimicrobials. In addition, patients who did not have stable lesions by roentgenography at the time of discharge and patients who were still producing tubercle bacilli were also discarded as inadequate treatment cases. Most of these patients were those who left the hospital against medical advice. Three hundred and eighty-seven cases were consequently discarded from the study, as shown in table 1, leaving 838 cases for study. These consisted of 412 males and 426 females. Their ages ranged from ten to more than seventy years, and the median age group was twenty-five to twenty-nine up to 1952, and thirty to thirty-four in subsequent years.

Follow-up Study

The routine procedure after discharge is to have all ex-patients return periodically for a follow-up review. These reviews are held at three sanatoriums and nine clinics stationed throughout the province of Saskatchewan; every patient is seen by a clinic doctor and has a chest roentgenogram. Reviews are recommended at three-month, six-month, or yearly intervals. While those who were discharged in 1946 had follow-up reviews up to the end of 1957 for eleven years, those who were discharged in 1955 had reviews for only two years.

Each year a lesser number of patients had follow-up reviews. The absentees include those who died from tuberculosis, those who died from non-tuberculous causes, those in whom relapse had been discovered, and those who did not attend because they had left the province or because of

[1] Fort Qu'Appelle Sanatorium, Saskatchewan Anti - Tuberculosis League, Saskatchewan, Canada.

[2] Excluded because of difficulty of follow-up observations.

TABLE 1

PATIENTS DISCARDED FROM STUDY

Discharge Year	Number of Patients Discharged	Reasons for Discarding			Total Discarded	Number of Patients Left for Study
		Inadequate Treatment	No Follow-up Study	Resection Surgery		
1946	137	36	3	0	39	98
1947	140	39	0	0	39	101
1948	119	22	1	0	23	96
1949	138	33	3	3	39	99
1950	123	42	6	3	51	72
1951	93	21	3	1	25	68
1952	112	20	4	4	28	84
1953	129	32	3	21	56	73
1954	137	29	2	18	49	88
1955	97	10	8	20	38	59
Total..	1,225	284	33	70	387	838

indifference. It may be thought that among those who failed to attend some may have suffered a reactivation or died from tuberculosis without the writer's knowledge, but this is not likely. The Saskatchewan Anti-Tuberculosis League is responsible for all tuberculosis work in the province. If a patient dies from tuberculosis, the Sanatorium is notified by the Vital Statistics Department and, if a patient has sought medical advice for tuberculosis, the Sanatorium is notified by his physician. Also, it is usually notified by other provinces if any of its ex-patients are having follow-up reviews by them. The only loophole is the patient not seeking medical attention and not attending the regular field survey which takes place periodically. Chest films of nearly all patients admitted to general hospitals in the province are taken at the request of the Saskatchewan Anti-Tuberculosis League.

Relapse

Relapse is considered to have taken place if there is bacteriologic or roentgenographic evidence of new or progressive disease or the development of extrapulmonary tuberculous disease. The estimated relapse percentage is computed by the life-table procedure. Only one relapse per case is counted, and that patient is no longer included among those whose disease might recur.

Chemotherapy

Virtually all patients discharged in 1946, 1947, and 1948 received no chemotherapy, and virtually all patients discharged in 1953, 1954, and 1955 did receive chemotherapy. In the intervening years, 1949 through 1952, some patients received no chemotherapy and some did receive chemotherapy for short periods. They are divided into the following four groups: no chemotherapy; chemotherapy for six weeks and more but less than six months; multiple-drug therapy (streptomycin and PAS) without isoniazid for six months and more; and isoniazid-streptomycin-PAS for six months and more.

Chemotherapy for a period of six weeks has been set as a convenient dividing line because in 1948, when streptomycin was first being used, it was customary to give intermittent courses of streptomycin, 1 gm. daily for six weeks comprising a course. Later on, PAS was administered along with streptomycin, and this was still given in short courses; but the streptomycin was given twice weekly. Finally, longer and continuous rather than interrupted treatment was given. Most of the 1953 dischargees were receiving continuous multiple-drug therapy for more than six months. From 1952 on, isoniazid was used, and again the period of treatment was increased. By 1955, all patients were having continuous multiple-drug therapy for at least one year. As a general rule the doses were: 1 gm. of streptomycin daily or twice weekly; 15 gm. of PAS daily; 300 mg. of isoniazid daily. Before streptomycin was given twice weekly it was preceded by streptomycin daily for three weeks.

RESULTS

Chemotherapy groups: In table 2 are shown the number of patients in the study who were discharged during the period of the study and the number who had had chemotherapy. Among the 838 patients, there were 425 who had had no chemotherapy, 191 who had had chemotherapy for six weeks or more but less than six months,

TABLE 2

CHEMOTHERAPY STATUS OF PATIENTS

Discharge Year	Number of Patients	Chemotherapy			
		None	Six Weeks or More, Less Than 6 Months	Six Months or More, Streptomycin-PAS	Six Months or More, Multiple Drugs Including Isoniazid
1946	98	98	0	0	0
1947	101	101	0	0	0
1948	96	94	2	0	0
1949	99	66	33	0	0
1950	72	32	40	0	0
1951	68	18	50	0	0
1952	84	14	59	11	0
1953	73	2	7	34	30
1954	88	0	0	15	73
1955	59	0	0	5	54
Total..	838	425	191	65	157

TABLE 3

PERIOD OF FOLLOW-UP STUDY AND CUMULATIVE RELAPSE RATE

(Estimated Percentage by Life-Table Procedure)

Chemotherapy	Year Discharged	Number of Patients and Total Cumulative Relapse	Cumulative Relapse Rate										
			Anniversary										
			1	2	3	4	5	6	7	8	9	10	11
None	1946–1953	425 (31.5%)	8.2	15.4	19.0	21.2	24.4	27.5	29.9	30.3	31.5	31.5	31.5
Six weeks or more, less than 6 months	1948–1953	191 (21.8%)	2.1	8.8	13.4	17.1	19.9	19.9	21.8	21.8	21.8		
Six months or more streptomycin-PAS	1952–1955	65 (12.5%)	3.4	3.4	5.8	12.5	12.5						
Six months or more multiple drugs including isoniazid	1953–1955	157 (8.8%)	2.1	6.0	8.8	8.8							

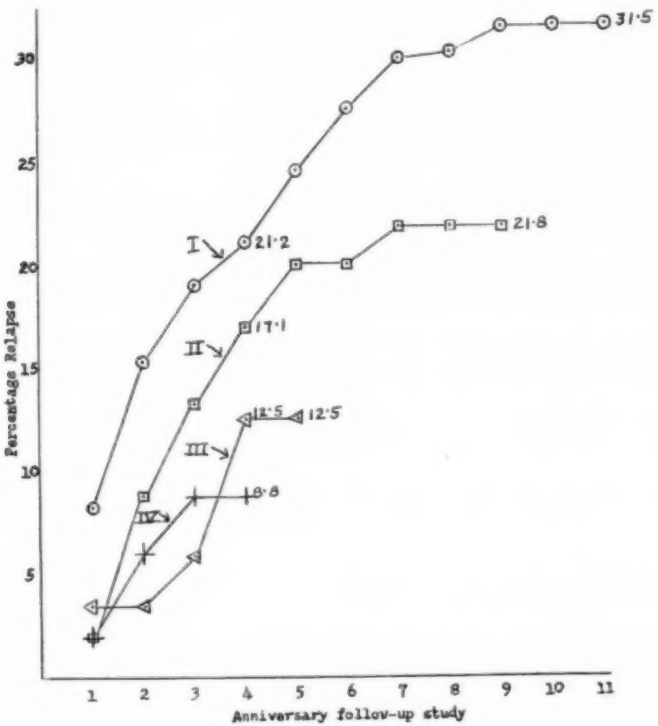

FIG. 1. Graph showing relapse rate in the four groups (percentage computed by life-table procedure).

I. ⊙—⊙ No chemotherapy—425 cases
II. ⊡—⊡ Chemotherapy 6 weeks to 6 months—191 cases
III. ◁—◁ Chemotherapy 6 months or more, streptomycin-PAS—65 cases
IV. +—+ Chemotherapy 6 months or more, multiple drugs, including isoniazid—157 cases

65 who received streptomycin-PAS for six months or more, and 157 who received multiple-drug therapy, including isoniazid, for six months or more.

Relapses: In table 3 and figure 1 the number of patients are shown according to their chemotherapy status, the period of follow-up study in years, and the cumulative relapse rate.

For the non-chemotherapy group, the cumulative relapse rate over an eleven-year follow-up period was 31.5 per cent. For the six-week-to-six-month group, the rate over a nine-year follow-up period was 21.8 per cent. For the streptomycin-PAS group, the rate over a five-year follow-up period was 12.5 per cent, and for the multiple-drug (including isoniazid) group, the rate over a four-year follow-up period was 8.8 per cent.

The relapse rate can be compared in the four groups only over a four-year follow-up period. The figures for such a period are, respectively, 21.2, 17.1, 12.5, and 8.8 per cent.

Relapse and extent of disease: The cases are separated into minimal, moderately advanced, and far advanced, according to National Tuberculosis Association standards (14) (table 4). Only three groups are shown: the non-chemotherapy group, the streptomycin-PAS group, and the multiple-drug (including isoniazid) group. The number of patients with cavitary disease on admission is also given. Previous to 1950, planigrams were not taken, but after 1950 planigrams were taken more frequently, and by 1955 all patients in whom cavitation was suspected had planigrams on admission. The median length of sanatorium stay for those who did not have chemotherapy and the mean average duration of chemotherapy for those treated with chemotherapy are given. The relapse rate for the patients with moderately advanced and far advanced disease is combined.

Analysis of relapses: In table 5 are listed the 15 patients treated with antimicrobials for six months or more in whom relapse occurred. Of these 15 patients, 9 had had initial chemotherapy of twice-weekly streptomycin-PAS, and 2 of these 9 had had interrupted courses; 2 had had isoniazid-streptomycin twice weekly, one had had daily isoniazid-PAS, one had had daily isoniazid-streptomycin, and 2 were started with streptomycin only and had had interrupted courses.

Eight patients showed roentgenographic progression only; 6, roentgenographic progression and positive bacteriologic findings; and one, positive bacteriologic findings without roentgenographic change. Eight of the 15 had cavitary disease on admission, and 7 had not.

TABLE 4

RELAPSE RATE ACCORDING TO EXTENT OF DISEASE: THREE GROUPS
(Estimated Percentage Computed by Life-Table Procedure)

Chemotherapy		Total	Minimal	Moderately Advanced	Far Advanced	Number Cavitary on Admission	Number of Relapses among Cavitary Cases
None	Number in study	425	207	119	99	70	20
	Median period in sanatorium, in months		12–15	15–18	30–33		
	Relapse rate, 11-year follow-up study		27.2%	35.3%			
Six months and more streptomycin-PAS	Number in study	65	25	27	13	13	3
	Mean average chemotherapy, in months	7.6	7.3	7.2	8.9		
	Relapse rate 5-year follow-up study		12.3%	12.3%			
Six months and more multiple drugs with isoniazid	Number in study	157	42	42	73	68	5
	Mean average chemotherapy, in months	15.2	12.4	13.3	17.9		
	Relapse rate, 4-year follow-up study		2.8%	11.1%			

TABLE 5

ANALYSIS OF 15 RELAPSES IN PATIENTS TREATED WITH ANTIMICROBIAL DRUGS FOR SIX MONTHS OR MORE

	Number of Patients	Year Discharged	Age on Discharge	Sex	Admission Bacteriologic Status and Roentgenographic Extent of Disease	Cavitary Disease on Admission	Combination of Chemotherapy in Months	Total Number of Months	Chemotherapy Continuous or Interrupted	Time of Relapse Months After Discharge
5 relapses among 65 patients treated with streptomycin-PAS	1	1953	21	F	B I	No	12S_2P	12	C	38
	2	1953	51	F	C I	No	10S_2P	10	C	42
	3	1953	51	M	B III	Yes	10S_2P	10	C	5
	4	1953	31	F	B II	Yes	7S_2P	7	Int.	48
	5	1953	34	F	B II	Yes	6S_2P	6	C	6
10 relapses among 157 patients treated with multiple drugs including isoniazid	6	1953	43	M	B III	Yes	7S_2P, 2H, 9S_2H	18	Int.	35
	7	1953	21	F	C II	No	4S_2P, 4S_2H	8	C	43
	8	1953	47	M	B III	Yes	1½S, 16S, 12S_2H	29½	Int.	20
	9	1953	40	F	B III	No	11S_2P, 6S_2H	17	C	15
	10	1954	20	M	B II	Yes	9S_2P, 5PH	14	C	20
	11	1954	32	F	C I	No	7PH	7	C	15
	12	1954	69	F	C III	No	8S_2H	8	C	25
	13	1954	52	M	C III	Yes	2SH, 5S_2H, 4PH	11	C	9
	14	1955	20	F	B III	No	10S, 2S_2P, 17S_2H	29	Int.	9
	15	1955	68	M	B III	Yes	2S_2H, 6S_2P, 9PH	17	C	7

I—minimal disease
II—moderately advanced disease
III—far advanced disease

B—tubercle bacilli were demonstrated; C—clinical cases, tubercle bacilli not having been demonstrated by microscopy or culture of sputum or gastric lavage

S—streptomycin only; S_2P—streptomycin twice weekly (daily for the first three weeks) plus PAS daily S_2H—streptomycin twice weekly (daily for the first three weeks) plus isoniazid daily; SH—streptomycin plus isoniazid daily; PH—PAS and isoniazid daily; C—continuous treatment; Int.—interrupted courses of treatment.

When more than one combination is given, the combination is arranged in chronologic order, the number in front of the letters representing the number of months of treatment.

DISCUSSION

The present series of cases contains four groups which permit a comparison of relapse incidence in patients treated without drugs, in patients treated with chemotherapy for less than six months, in patients treated for at least six months with streptomycin-PAS, and in patients treated for at least six months with a multiple-drug regimen containing isoniazid. They had similar institutional care, although over different years, and they are similar in respect to race, age, and sex. Resection cases are not included. Among the patients treated up to 1952 there was a larger proportion who had artificial pneumothorax or pneumoperitoneum, and also a larger proportion had thoracoplasties in these years. In all other respects the groups appear to be similar. However, there is an important difference in that the discharged patients in the non-chemotherapy group represent the more hardy ones who had "been through the mill" and had survived, manifesting greater resistance to the disease and better response to the form of treatment without drugs. Those who were discharged following chemotherapy include many less hardy ones, with less resistance, who no doubt would have succumbed without the assistance of drugs. This may be evidenced by the fact that before the introduction of drugs one of five discharges was by death from tuberculosis (2), and a far less proportion were discharged as "stable." Other workers have attempted to correlate relapse with the state of pre-existing cavity or cavities at the time of discharge, but in this study an attempt is made to correlate relapse with the kind and duration of chemotherapy the patient received.

Among the 425 non-chemotherapy cases, the cumulative relapse rate over an eleven-year

follow-up period was 31.5 per cent. About 50 per cent of these relapses took place in the first two years; about 70 per cent in the first four years; and after the sixth year the relapse rate was insignificant. This agrees with the findings of most observers, including work done previously in this institution (1, 2). The relapse rate was greater in the advanced cases than in the minimal cases. This is not in accordance with the findings at Trudeau Sanatorium where the relapse incidence was the same in the patients with minimal disease as in the other groups (3, 4).

There were 191 patients discharged from 1948 through 1953 who had chemotherapy for less than six months, some of them single-drug therapy, usually streptomycin, which is now considered to be inadequate. The cumulative relapse rate among them over a nine-year follow-up period of 21.8 per cent is less than among those who did not have chemotherapy. Before drugs were being given to all patients, it was the practice to give drugs only to those patients who were more seriously ill, so, compared with patients who were discharged at the same time and did not receive antimicrobial drugs, this was a selected less favorable group. The lower relapse rate in this group compared with that in the non-chemotherapy group is more significant than is indicated by the figures.

There were 65 patients who had therapy with streptomycin-PAS for six months or more. These patients were discharged from 1952 through 1955 in practically the same period as those who received multiple-drug therapy containing isoniazid. This streptomycin-PAS group, compared with the isoniazid-containing multiple-drug group, consisted of the less seriously ill patients or the patients who were responding favorably to treatment; they were a selected group, a more favorable group. It may be seen in table 4 that in this streptomycin-PAS group there were 20 per cent far advanced and 20 per cent cavitary cases, while in the isoniazid group there were 46.5 per cent far advanced and 43.3 per cent cavitary cases. Even so, the relapse rate in the group of 12.5 per cent is higher than the 8.8 per cent in the isoniazid-containing multiple-drug group.

The relapse rate in the isoniazid group of 157 patients was 8.8 per cent over a four-year follow-up period. The total number of relapses was 10: 8 of these in the first two years, 2 in the third year, and none in the fourth year. Some European observers have noted that, in patients treated with chemotherapy, relapses tend to appear in the third year rather than in the first two years, as occurs among those treated without antimicrobials (15). They also noted a reduction in relapse after three years, and the same has been observed at the Veterans Hospital at Sunmount, New York (11). This is not seen in the isoniazid-containing multiple-drug group, but such a tendency is seen in the streptomycin-PAS group.

Patients in the isoniazid group were discharged in the years 1953 through 1955, and those discharged in 1955 had longer periods of chemotherapy than those discharged in earlier years. In 1955 these patients had been receiving drug therapy for at least one year and often for fifteen to eighteen months. Of the 10 relapses that appeared in this group, only 2 occurred in the 54 patients discharged in 1955, and one of these patients had had initial treatment with streptomycin only and had had intermittent courses of chemotherapy. If this case is excluded, the relapse incidence for the 1955 dischargees is about 2 per cent. The optimal duration of chemotherapy required in the treatment of tuberculosis is not known. It has been stated that, after prolonged chemotherapy with isoniazid-containing multiple-drug therapy, the persistence of "viable" tubercle bacilli is rare, but figures vary from 2 to 64 per cent (16–20). Ross claims that he and his colleagues in the Edinburgh Tuberculosis Service "cannot recall a single patient with initially sensitive organisms and receiving daily streptomycin and INH in whom they observed or had reason to suspect drug resistance" (21). He and his co-workers report not one relapse among 63 patients with susceptible organisms treated for more than eighteen months (13). In this institution, susceptibility studies on the bacilli were not being done routinely on admission, but in those cases in which such studies were made the bacilli were invariably susceptible to the three standard drugs, and the 5 per cent of patients with bacilli resistant to one of the three standard drugs used in the United Kingdom is not seen here (22). Domiciliary treatment with drugs is not practiced except after stabilization of the lesion in the sanatorium. Since the reports of the Medical Research Council in November, 1953, and February, 1955 (23, 24), indicating the superiority of isoniazid-streptomycin daily rather than twice weekly in delaying the emergence of or-

ganisms resistant to isoniazid, many of the patients who started their treatment in 1954 had had isoniazid-streptomycin daily as their initial treatment. It may be seen in table 5 that, among the 10 relapses in patients who received multiple-drug therapy including isoniazid, only one of these was in a patient whose initial treatment was with isoniazid-streptomycin daily, and he had had drug therapy for only eleven months. With regard to the duration of drug therapy, it is also shown in table 4 that, while the patients with minimal disease on multiple-drug therapy including isoniazid did fairly well—2.8 per cent relapse—the patients with advanced disease who had drug therapy for twelve months did not do well—11.1 per cent relapse—with drug therapy for thirteen to eighteen months. However, all relapses with one exception were in patients not treated initially with isoniazid-streptomycin.

In patients with cavitary disease, there was quite a difference in the relapse incidence in those treated with streptomycin-PAS and those treated with an isoniazid-containing combination. The former group showed 3 relapses among 13 patients; and the latter group, 5 relapses among 68 patients. But the streptomycin-PAS group had chemotherapy for an average of 7.6 months; and the group treated with multiple drugs including isoniazid, for an average of 15.2 months. The numbers, of course are too small for any definite conclusions to be drawn.

The above findings would indicate that patients do better with initial treatment with isoniazid and daily streptomycin and that, in patients with cavitary disease, treatment with multiple drugs containing isoniazid is essential. Patients with minimal disease may do better with drug therapy for more than twelve months; and those with advanced disease, for more than eighteen months.

This study does not include patients who had resectional surgery, but does include those who were rejected for surgery or who were not even considered for surgery because of poor general condition, inadequate pulmonary function, or widespread bilateral disease. The relapse rate in the isoniazid group, including such cases, is 8.8 per cent. The mortality and morbidity figures from the surgical department for resections performed between 1955 and 1957 are, respectively, 3.6 and 21.4 per cent (25), and figures from other centers are in the same range (12, 26–28). The relapse incidence in resected cases of closed necrotic lesions has been reported as being not significantly different from that in nonresected cases (12, 16, 29). Stewart and her co-workers claim that "provided the organisms are sensitive to the drugs in use, chemotherapy can sterilize both cavities and caseous foci irrespective of size" (17). In view of these findings, it would seem that the definitive management by medical rather than by surgical measures is to be preferred in cases of closed necrotic lesions that are stable.

The relapse rate of 8.8 per cent in the isoniazid group is not very different from that reported by other observers over a similar period. Ross and his colleagues quote the figure of 7 per cent in 264 patients followed for three to four years up to the end of 1956. It is hoped that for the dischargees after 1955 there will be a much lower relapse rate.

After the material for this study had been compiled, data were gathered on the 1956 dischargees, followed to the end of June, 1958, who had had continuous chemotherapy for at least fifteen months. There was no relapse in 30 cases, and among 80 patients discharged from 1954 through 1956 after a similar duration of chemotherapy there has been only one relapse, already reported in this study; this was in a patient whose initial chemotherapy was isoniazid-streptomycin twice weekly.

The drop in relapse incidence from 21.2 per cent over a four-year follow-up period in pre-chemotherapy days to 8.8 per cent in the 1953–1955 dischargees treated with antimicrobial drugs is encouraging, but can certainly be improved upon. This drop has been brought about by treatment which is considered to be inadequate in most of the cases today. In the group treated with antimicrobials for more than six months, it is not considered that any of the 15 patients in whom relapse occurred had had adequate chemotherapy. It is anticipated that, with the present routine of initial isoniazid-streptomycin daily until sputum "conversion" has taken place and if chemotherapy is continued for eighteen months or longer, the relapse incidence in original treatment cases with bacilli susceptible to the three standard drugs will not be more than 1 to 2 per cent.

Summary

A study was made of the relapse incidence in 838 patients with pulmonary tuberculosis who were discharged from the Fort Qu'Appelle Sana-

torium from 1946 through 1955. Only disease considered to be stable both roentgenographically and bacteriologically, and not necessitating resection surgery, is included.

The patients are placed in four separate groups: those treated without drugs, those who had six weeks or more but less than six months of chemotherapy, those who received streptomycin-PAS for six months or more, and those who received multiple-drug therapy including isoniazid for six months or more.

The cumulative relapse rate, computed by life-table procedure, is given for each group. The follow-up period is from two to eleven years. Over a follow-up period of four years computed for all of the groups, the cumulative rate is 21.2 per cent for the non-chemotherapy group, 17.1 per cent for the group which received chemotherapy from six weeks to six months, 12.5 per cent for the group which received streptomycin-PAS for six months and longer, and 8.8 per cent for the group which received multiple-drug therapy containing isoniazid for six months and more.

The relapses following multiple-drug chemotherapy for six months and more are analyzed with relation to extent of disease and to the combination and duration of chemotherapy.

Sumario

La Tasa de Recidivas en un Período de Dos a Once Años de Observación de Casos de Tuberculosis Pulmonar Tratados con y sin Quimioterapia y Dados de Alta desde 1946 Hasta 1955 Inclusive

Este estudio comprende la incidencia de recidivas en 838 enfermos de tuberculosis pulmonar que fueron dados de alta del Sanatorio Fort Qu'Appelle desde 1946 hasta 1955 inclusive. Sólo figura en él la enfermedad considerada como estable tanto radiográfica como bacteriológicamente y que no necesitó la resección quirúrgica.

Los enfermos aparecen distribuídos en cuatro grupos distintos: los tratados sin drogas, los que recibieron seis semanas o más pero menos de seis meses de quimioterapia, los que recibieron estreptomicina-PAS durante seis meses o más y los que recibieron polifarmacoterapia, isoniacida inclusive, durante seis meses o más.

Se presenta para cada grupo la tasa acumulativa de recidivas, computada conforme a los procedimientos de las tablas de demografía. El período de observación es de dos a once años. Durante un período de observación de cuatro años, computado para todos los grupos, la tasa cumulativa es de 21.2 por ciento para el grupo sin quimioterapia, 17.1 por ciento para el grupo que recibió quimioterapia de seis semanas a seis meses, 12.5 por ciento para el que recibió estreptomicina-PAS durante seis meses o más y 8.8 por ciento para el que recibió polifarmacoterapia con isoniacida durante seis meses o más.

Las recidivas consecutivas a la quimioterapia combinada durante seis meses y más son analizadas con relación a la difusión de la enfermedad y a la combinación y duración de la quimioterapia.

Resume

Taux de rechute enregistré durant une période d'observation de deux à onze ans de cas de tuberculose pulmonaire soumis ou non à la chimiothérapie et sortis de l'hôpital de 1946 à 1955 inclus

Il a été procédé à une étude de l'incidence des rechutes chez 838 tuberculeux pulmonaires sortis du Sanatorium de Fort qu'Appelle de 1946 à 1955 inclus. L'étude comporte uniquement les cas considérés stables à la fois des points de vue radiologique et bactériologique et ne nécessitant pas la chirurgie d'exérèse.

Les malades sont répartis en quatre groupes séparés: ceux traités sans médicaments; ceux ayant été soumis à la chimiothérapie pendant six semaines ou plus, mais moins de six mois; ceux ayant été traités par l'association streptomycine-PAS pendant six mois ou plus, et ceux ayant été soumis à un traitement combiné comportant l'isoniazide pendant six mois ou plus.

Un taux cumulatif de rechute, calculé selon la méthode de la table de mortalité est donné pour chaque groupe. La période d'observation est de deux à onze ans. Les déterminations effectuées durant une période d'observation de quatre ans pour tous les groupes, indiquent un taux cumulatif de 21, 2% pour le groupe non soumis à la chimiothérapie, de 17,1% pour le groupe soumis à la chimiothérapie pendant six semaines à six mois, de 12, 5% pour le groupe traité avec l'association streptomycine-PAS pendant six mois et plus et de 8, 8% pour le groupe recevant pendant six mois et plus le traitement combiné comprenant l'isoniazide.

Une analyse des rechutes à la suite de la chimiothérapie combinée pendant six mois et plus par rapport à l'étendue de la maladie, à l'association antibiotique utilisée et à la durée de la chimiothérapie a été effectuée.

Acknowledgment

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SEROLOGIC RELATIONSHIP OF OLD TUBERCULIN-SENSITIZED SHEEP ERYTHROCYTES AND TRYPSINIZED HUMAN ERYTHROCYTES[1,2]

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INTRODUCTION

The deliberate treatment of erythrocytes with biologic agents, their products, or with better-defined chemical substances in order to confer additional antigenic specificities to the erythrocyte surface is accepted procedure in present-day serology.

Previous investigations from this laboratory have been concerned with modification of mammalian erythrocytes by treatment with Old Tuberculin (OT) or with aqueous extracts of bacillary masses of various mycobacteria (1–4). Erythrocytes so treated have been used principally for the detection of antibodies in the serum of animals experimentally infected with tuberculosis or injected with other mycobacteria.

During the course of these investigations, it was noted that human erythrocytes treated with extracts from BCG failed to demonstrate hemosensitizing antigens by direct tests (hemolysis or hemagglutination) with specific antiserums (5). Their presence on the erythrocytes, however, was detected by adsorption experiments. Furthermore, a marked difference was observed in the susceptibilities to hemolysis of OT-sensitized human and sheep erythrocytes when tested with the same antiserums in the presence of guinea pig complement.

These findings prompted further studies on the antigenic modifications demonstrated by treated sheep erythrocytes with particular reference to their immunogenicity. Rabbit erythrocytes were subjected to similar experimentation.

A corollary investigation was initiated to determine if the specificities of tuberculin-treated erythrocytes, as demonstrated with antimycobacterial serums, comprise the total antigenic modifications of these erythrocytes or whether additional changes, not readily measured, are created by the adsorption of tuberculin products to the erythrocyte surface. The obvious need for another type of modified erythrocyte for purposes of comparison resulted in the selection of the trypsinized human erythrocyte. The proper enzymic treatment of these erythrocytes results in a detectable antigenic alteration of the erythrocyte surface.

The specific results of the experiments outlined above are the subject of this paper.

MATERIALS AND METHODS

Sensitization of erythrocytes with Old Tuberculin: Sheep or rabbit erythrocytes were separated from citrated blood by centrifugation and were thrice washed with phosphate-buffered physiologic saline (pH 7.4). The procedure for sensitization has been reported previously from these laboratories (6) and consisted of mixing 60 volumes of 1:15 OT[3] with one volume of washed, packed erythrocytes, followed by incubation for two hours at 37°C. (water bath). After removing the excess OT by repeated centrifugation with changes of phosphate-buffered saline, the sensitized erythrocytes were either diluted to the proper concentration for serologic testing (0.5 per cent) or maintained in the packed state for adsorption studies.

Trypsinization of human erythrocytes (7)*:* Group *O* human erythrocytes were collected and washed in the usual manner. A stock solution of 1.0 per cent trypsin (Difco 1:250) was prepared in phosphate-buffered saline (pH 7.4). This stock was further diluted to 0.1 per cent, 1.5 volumes of which were added to 1.0 volume of erythrocytes, and the mixture was incubated for ten minutes at 37°C. (water bath). After washing with saline, these enzyme-modified erythrocytes were suspended to 0.5 per cent for serologic testing or to 5.0 per cent for purposes of injection. Packed enzyme-treated erythrocytes were retained for adsorption procedures.

Immunization of animals: Young adult rabbits weighing 2 to 3 kg. each were injected intravenously with 0.02 mg. of viable *Mycobacterium tuberculosis* var. *bovis* (Ravenel) or with 1.0 mg. of viable BCG. These organisms had been cultivated on Löwenstein's medium at 37°C. until adequate growth was observed, and were then

[1] From the Department of Bacteriology, Ohio State University, Columbus, Ohio.

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[3] Old Tuberculin Lederle (4X International Standard) was generously supplied by Dr. W. S. Hammond, Lederle Laboratories Division, American Cyanamid Co., Pearl River, New York.

harvested into weighing bottles. Sufficient saline was added to effect the desired weight of the inocula per milliliter.

Trypsinized erythrocytes and erythrocytes modified by sensitization with OT were also employed for injection into rabbits. The details of the immunizations will be discussed under the appropriate experiments.

Serums: All animals were bled by cardiac puncture prior to receiving any treatment. These serums and those recovered from postinjection blood samples were maintained in the frozen state (−20°C.) until needed. Before testing, all serums were inactivated at 56°C. for thirty minutes and then adsorbed twice (twenty to thirty minutes per adsorption, room temperature) with quarter volumes of packed sheep and human erythrocytes. These adsorptions completely removed the naturally occurring antibodies to these erythrocytes.

Additional adsorptions with the modified erythrocytes were conducted according to the protocol of the particular experiment.

Hemagglutination and hemolytic tests: The techniques for performing these tests were essentially those described previously (6) and consisted of "drop" modifications of generally accepted methods. Doubling dilutions of the test serums were prepared with buffered saline so that the final volume of each dilution was 2 drops. An equal quantity of the appropriate erythrocyte antigen was added to each serum dilution, after which the tests were incubated at 37°C. (water bath) for thirty minutes. Hemagglutination titers were determined as the last serum dilution exhibiting readily discernible agglutination after light centrifugation (1,000 r.p.m. in an angle centrifuge for one minute).

Hemolytic tests were conducted in a similar manner except that 1 drop of 1:9 guinea pig complement, previously reconstituted from the dehydrated state and adsorbed twice in the cold with packed erythrocytes, was added to each tube in the test. The end point of an hemolytic titration was considered as the last serum dilution demonstrating complete hemolysis.

Proper antigen and serum controls were included with each test. Particular attention was paid to the trypsinized human erythrocyte antigen since over-trypsinization results in a tendency toward pan-agglutinability, even with saline.

Experimental Results

Immunogenicity and in vitro specificity of OT-treated sheep and rabbit erythrocytes:

Both sheep and rabbit erythrocytes were sensitized with OT as described above. Groups of normal rabbits were injected intravenously with 5.0 ml. of a 0.5 per cent suspension of one or the other of these antigens at three-day intervals for a total of three injections. Blood samples were procured by cardiac puncture on the fifth and twelfth days after the last injections, and the serums inactivated (56°C., thirty minutes), adsorbed with normal cells and tested with either OT-treated sheep or rabbit erythrocytes before and after cross-adsorptions, according to the procedures indicated in table 1.

The results shown in the table are typical of those obtained from replicate experiments. In no instance was hemolysis evident when serums were tested with OT-sensitized rabbit erythrocytes, employing adsorbed guinea pig complement diluted 1:9 (*see* Materials and Methods). Increasing the concentration of complement had no effect on these results. When used in the undiluted state, an occasional serum dilution exhibited partial hemolysis. Invariably, these reactions occurred only in the first or second test dilutions (1:2, 1:4). Despite the failure to demonstrate the presence of the lytic antigen(s) on the surface of the rabbit erythrocyte by direct tests (addition of guinea pig complement), adsorption of serums with the treated rabbit erythrocytes completely removed the lytic antibodies measurable with the OT-sensitized sheep erythrocytes. Reciprocal adsorptions with OT-sensitized sheep erythrocytes also removed all detectable antibodies. Generally, little or no difference was noted in the hemagglutination titers obtained with either type of sensitized erythrocyte antigen. These results are not unlike those observed previously with sensitized human erythrocytes (5, 8, 9).

Of particular interest was the antibody response of rabbits following injections of the OT-sensitized erythrocyte antigens. It would appear that the rate at which antibodies to OT were produced varied with the erythrocyte carrier. The antibody titers to the OT-treated sheep erythrocytes were maximal in the fifth-day serum samples and were diminished slightly in the twelfth-day samples. Conversely, antibodies to the OT-sensitized rabbit erythrocytes appeared in highest titers in the twelfth-day samples.

For purposes of comparison, OT was injected into rabbits without benefit of erythrocyte carriers and the antibody response was measured as described below.

Antibody titers of rabbits injected with Old Tuberculin:

Old Tuberculin, diluted 1:15 with phosphate-buffered saline (pH 7.4), was injected intravenously into rabbits with no further treatment

TABLE 1

IMMUNOGENICITY AND IN VITRO SPECIFICITY OF OT-SENSITIZED SHEEP AND RABBIT ERYTHROCYTES

Antiserums to:	Adsorbed with:	Days post Injection	Titers When Tested with: OT-sensitized Sheep Erythrocytes HL*	OT-sensitized Sheep Erythrocytes HA	OT-sensitized Rabbit Erythrocytes HL	OT-sensitized Rabbit Erythrocytes HA
OT-sensitized sheep erythrocytes	Normal sheep erythrocytes	5	512	256	—	256
		12	256	64	—	64
	OT-sensitized sheep erythrocytes	5	—	— †	—	—
		12	—	—	—	—
	OT-sensitized rabbit erythrocytes	5	—	—	—	—
		12	—	—	—	—
OT-sensitized rabbit erythrocytes	Normal sheep and rabbit erythrocytes	5	32	4	—	32
		12	256	64	—	64
	OT-sensitized sheep erythrocytes	5	—	—	—	—
		12	—	—	—	—
	OT-sensitized rabbit erythrocytes	5	—	—	—	—
		12	—	—	—	—

* HL refers to hemolysis; HA to hemagglutination. Titers expressed as reciprocals of serum dilutions.

† [ ] Indicates homologous adsorption.

or after adsorption with normal sheep or rabbit erythrocytes. Each animal received three injections of 1.0 ml. (per injection) spaced at three-day intervals. As before, the animals were bled on the following fifth and twelfth days and the serums were tested with OT-sensitized sheep and rabbit erythrocytes.

The highest titers were found in twelfth-day serums and are recorded in table 2. Obviously, the prior adsorption of OT with either species of erythrocyte successfully removed all of the erythrocyte-adsorbable antigens since antiserums to these antigens failed to react with either type of sensitized erythrocyte in the *in vitro* tests. The antibody titers observed with the serums of rabbits which had received the unadsorbed OT were significantly lower than were those observed following the injection of the OT-treated erythrocytes, particularly the lytic antibody demonstration.

Specificities of trypsinized human erythrocytes: On occasion, it has been noted in this laboratory that rabbit-immune serums developed to various antigens reacted to low titers with OT-sensitized sheep erythrocytes despite adsorptions with normal erythrocytes from this species. These reactions suggested that treatment of the sheep erythrocyte with OT may have created a speci-

TABLE 2

SERUM TITERS OF RABBITS INJECTED WITH UNADSORBED AND ADSORBED OLD TUBERCULIN

Antiserums to OT: Treatment of Immunizing Agent (OT)	Test Antigen: OT-sensitized Sheep Erythrocytes HL	OT-sensitized Sheep Erythrocytes HA	OT-sensitized Rabbit Erythrocytes HL	OT-sensitized Rabbit Erythrocytes HA
Untreated	32	16	—	32
Previously adsorbed with normal sheep erythrocytes	—	—	—	—
Previously adsorbed with normal rabbit erythrocytes	—	—	—	—

ficity additional to that usually attributable to the adsorbed tuberculin.

Since many normal serums exhibit minimal reactivity to trypsinized human erythrocytes, this particular erythrocyte modification was arbitrarily selected for study.

Serums collected at intervals from rabbits injected intravenously with several of the mycobacteria were inactivated and adsorbed with both normal sheep and human erythrocytes to remove the naturally occurring agglutinins. These serums, including preinjection (or baseline) serums, then were tested with OT-sensitized sheep erythrocytes and with trypsinized human erythrocytes. Other samples of the same serums were subjected to reciprocal adsorptions with one or the other of the modified erythrocytes and were subsequently tested with both the homologous and heterologous modified erythrocyte antigens. The results obtained from testing anti-*M. bovis* and anti-BCG serums are recorded in tables 3 and 4. These data were selected for presentation since they are typical of those obtained from testing other antimycobacterial serums.

The hemolysin and hemagglutinin titers of the serums as measured with OT-sensitized sheep erythrocytes were in keeping with those generally encountered in this laboratory. These titers were

TABLE 3

BCG Serums: Influence of Reciprocal Adsorptions on In Vitro Reactivity

Test Serum	Weeks post Injection	Antibody Titer to:											
		OT-sensitized Sheep Erythrocytes*						Trypsinized Human Erythrocytes†					
		Unadsorbed		Homologous Adsorption		Heterologous Adsorption		Unadsorbed		Homologous Adsorption		Heterologous Adsorption	
		HL	HA	HL	HA	HL	HA	HL	HA	HL	HA	HL	HA
Anti-BCG	Preinjection	—	—	—	—	—	—	—	2	—	—	—	—
	1	256	8	—	—	32	8	—	2	—	—	—	—
	3	512	64	—	—	256	64	—	4	—	—	—	—
	4	512	64	—	—	128	32	—	4	—	—	—	—
	5	128	16	—	—	64	16	—	4	—	—	—	—
	8	32	16	—	—	32	8	—	4	—	—	—	—

* Homologous adsorption with OT-sensitized sheep erythrocytes; heterologous adsorption with trypsinized human erythrocytes.

† Homologous adsorption with trypsinized human erythrocytes; heterologous adsorption with OT-sensitized sheep erythrocytes.

TABLE 4

Anti-M. Bovis Serums: Influence of Reciprocal Adsorptions on In Vitro Reactivity

Antiserums to:	Weeks post Injection	Antibody Titer to:											
		OT-sensitized Sheep Erythrocytes*						Trypsinized Human Erythrocytes*					
		Unadsorbed		Homologous Adsorption		Heterologous Adsorption		Unadsorbed		Homologous Adsorption		Heterologous Adsorption	
		HL	HA	HL	HA	HL	HA	HL	HA	HL	HA	HL	HA
M. bovis	Preinjection	—	—	—	—	—	—	—	2	—	—	—	—
	1	16	4	—	—	8	2	—	2	—	—	—	—
	4	512	8	—	—	256	16	—	2	—	—	—	—
	5	256	4	—	—	128	8	—	2	—	—	—	—
	7	256	4	—	—	128	4	—	4	—	—	—	—

* Adsorptions the same as in table 3.

relatively uninfluenced by multiple adsorptions with packed trypsinized human erythrocytes. However, the testing of the unadsorbed serums with a 0.5 per cent suspension of the enzyme-treated erythrocytes resulted in hemagglutination in the lower serum dilutions (1:2 to 1:4). This reactivity was demonstrated with both pre- and postinjection serums, no significant increase being associated with the later serum samples. In no instance was hemolysis observed upon the addition of 1:9 complements to the system.

Adsorption of serums with packed OT-treated sheep erythrocytes removed these hemagglutinins, thus suggesting that at least one specificity is held in common by the two types of modified erythrocytes.

The number of adsorptions with OT-treated erythrocytes necessary to remove the trypsinized erythrocyte antibody varied markedly among the test serums; some required four to five adsorptions with equal quantities of packed erythrocytes to effect complete antibody removal. This was especially true of the higher titered antimycobacterial serums and indicated that OT antibodies, when present in serums containing hemagglutinins to trypsinized human erythrocytes, were preferentially removed by adsorption with OT-treated sheep erythrocytes.

The serums prepared to OT and to OT-modified sheep and rabbit erythrocytes were tested as were the tuberculous serums (table 5), with precisely the same results. All serums contained hemagglutinins to trypsinized erythrocytes which were adsorbable with OT-sensitized sheep erythrocytes. The reverse adsorption had no effect on the demonstrations with the tuberculin-treated erythrocytes.

An antiserum to the trypsinized erythrocyte was produced by repeatedly injecting a rabbit intravenously with suspensions of enzyme-modified human erythrocytes. The resulting serum titrated with the homologous antigen to a dilution of 1:33,760, and with the OT-sensitized sheep erythrocyte antigen, to a dilution of 1:4. Hemagglutination was the only reaction observed. Adsorptions with trypsinized erythrocytes, sufficient to lower the titer of the homologous antibodies to 1:64, completely removed the antibodies to the tuberculin-treated sheep cells.

Sixteen serums from rabbits injected intravenously with a variety of antigens were properly adsorbed and examined for substances reactive with OT-sensitized sheep erythrocytes and for antibodies to trypsinized human erythrocytes. Although these animals had received multiple injections of their respective antigens, none had been introduced with benefit of any adjuvant.

All serums tested reacted with both OT-treated sheep erythrocytes and enzyme-modified human erythrocytes in relatively low titers (table 6). Five normal rabbit serums, included as controls, reacted only with the trypsin-treated erythrocytes, as anticipated. It is of interest that reac-

TABLE 5

SERUMS TO OT-SENSITIZED ERYTHROCYTES: INFLUENCE OF RECIPROCAL ADSORPTIONS ON IN VITRO REACTIVITY

Antiserums to:	Days post Injection	Antibody Titer to:											
		OT-sensitized Sheep Erythrocytes*						Trypsinized Human Erythrocytes*					
		Unadsorbed		Homologous Adsorption		Heterologous Adsorption		Unadsorbed		Homologous Adsorption		Heterologous Adsorption	
		HL	HA	HL	HA	HL	HA	HL	HA	HL	HA	HL	HA
OT-sensitized sheep erythrocytes	5	512	256	—	—	256	256	—	4	—	—	—	—
	12	256	64	—	—	128	64	—	4	—	—	—	—
OT-sensitized rabbit erythrocytes	5	32	4	—	—	32	4	—	4	—	—	—	—
	12	256	64	—	—	64	64	—	2	—	—	—	—
OT	5	32	16	—	—	16	16	—	4	—	—	—	—

* Adsorptions the same as in table 3.

TABLE 6

REACTIVITY OF VARIOUS ANTISERUMS TO OT-SENSITIZED SHEEP AND TRYPSINIZED HUMAN ERYTHROCYTES, AND THE INFLUENCE OF RECIPROCAL ADSORPTIONS

Antiserums to:	Antibody Titer to:											
	OT-sensitized Sheep Erythrocytes*						Trypsinized Human Erythrocytes*					
	Unadsorbed		Homologous Adsorption		Heterologous Adsorption		Unadsorbed		Homologous Adsorption		Heterologous Adsorption	
	HL	HA	HL	HA	HL	HA	HL	HA	HL	HA	HL	HA
E. coli "O"	8	16	—	—	—	4	—	16	—	—	—	4
E. coli "OB"	64	16	—	—	64	8	—	4	—	—	—	—
Sheep serum	16	4	—	—	—	—	—	8	—	—	—	—
Brucella spp	8	2	—	—	—	—	—	2	—	—	—	—
Hapamine	8	—	—	—	—	—	—	16	—	—	—	—
Sal. typhosa "O"	8	2	—	—	—	—	—	32	—	—	—	—
Sal. typhosa "H"	64	32	—	—	—	4	—	4	—	—	—	—
Sal. paratyphi	8	4					—	2				
Swine serum	—	2					—	8				
Human erythrocytes	8	4					—	8				
Sheep erythrocytes	—	8					—	4				
Mumps virus	8	+†					—	2				
Ovalbumin	8	2					—	2				
Human serum	8	+					—	16				
Horse serum	8	4					—	8				
Forssman	8	2					—	4				

* Adsorptions the same as in table 3.
† Indicates undiluted serum.

tivity to the tuberculin-treated erythrocytes was not confined to a single demonstration. Some test systems exhibited both lysis and hemagglutination. Adsorption of the serums with trypsinized human erythrocytes removed the demonstrable antibodies to tuberculin-treated erythrocytes except in those antiserums prepared to *Escherichia coli*. These results bear out the contention, other than in the instance just mentioned, that both types of modified erythrocytes share a common antigenic specificity.

DISCUSSION

Under certain experimental situations, it is possible to produce specific antibodies to erythrocyte-adsorbable antigens by the injection of the treated erythrocyte (10, 11). The resulting antiserum will also contain antibodies to the "carrier" erythrocyte, providing the erythrocyte was from a heterologous species or from the same species but of an incompatible type. From the data presented here, it is obvious that antibodies to the erythrocyte-adsorbable components of OT can be elicited by injecting rabbits with OT-treated sheep or rabbit erythrocytes. Differences were noted, however, in the rate and titer of the antibody production and may reflect the stimulatory threshold of the antibody-forming mechanism of the rabbit. The antibodies to OT attained higher levels earlier if the antigen was adsorbed to sheep erythrocytes prior to injection than if the rabbit erythrocyte served as the "carrier." In the former instance, it is possible that the rabbit responded more actively, or to a greater extent, to the completely foreign sheep erythrocyte and concomitantly to the adsorbed OT. The titers to the OT-sensitized rabbit erythrocyte, observed under the conditions of the experiment, may have represented, moreover, an exaggerated response since no effort was made to select erythrocytes for OT treatment of the same blood type as were the recipient rabbits. During the course of the investigation, iso-antibodies (hemagglutinins) in titers to 1:128 sometimes were encountered, but were eliminated from confounding testing procedures by proper adsorptions. However, the possible stimulatory effect of the rabbit iso-antigens may have influ-

enced the titers developed to the adsorbed OT antigens.

In any event, the injection of OT when adsorbed to either species of erythrocyte resulted in higher levels of antibodies than did the injection of OT alone. Further, adsorption of the OT with these erythrocytes before injection apparently removed a sufficient quantity of antigen so that no serum antibody was detectable (as stated before).

The value of the erythrocyte carrier in the production of antibodies to OT is emphasized by the fact that the quantity of unadsorbed OT introduced into each animal (1.0 ml. of 1:15 OT) was much greater than could be adsorbed onto the surfaces of the erythrocytes which were employed for injection. It has been shown by experimentation in this laboratory (1), as well as in other laboratories, that erythrocyte-adsorbable antigens remain in the supernatant fluid of 1:15 OT after a single adsorption with normal sheep erythrocytes, as in the preparation of the *in vitro* antigen.

The failure to demonstrate hemolysis of the OT-sensitized rabbit erythrocyte in the presence of specific antiserums and guinea pig complement evidently is due to species refractoriness and has been reported previously for erythrocytes from other species (8, 12, 13).

The treatment of certain erythrocytes with a variety of agents, among them viruses (14–16), periodate (17, 18), or chromate ions (19), and bacterial (16, 20–22) or proteolytic enzymes (22–25) often results in modifying the antigenic character of the erythrocyte surface. In some instances, these variously modified erythrocytes share common antigenic specificities, as with the trypsinized and the influenza virus-treated human erythrocyte (26).

In the studies reported here, antiserums to a number of immunizing substances, as well as the antimycobacterial serums but not normal rabbit serums, were found to be reactive with OT-treated erythrocytes. The antibodies in the nonmycobacterial antiserums were of low titers and were adsorbable in most instances by trypsinized human erythrocytes. The antiserums which retained their specificity for the erythrocyte-adsorbable components of OT were from animals injected either with mycobacteria or with bacteria known to contain antigenic carbohydrates. This suggests a special relationship between these substances present in the enteric organisms and the polysaccharides of OT.

All rabbit serums, whether from immunized or normal animals, agglutinated trypsinized human erythrocytes but to low titers only, with the exception of the hyper-immune trypsin-cell serum. In all but the latter instance, these antibodies were readily removed by adsorption with OT-modified sheep erythrocytes.

This naturally occurring antibody resembles in many respects the "T" agglutinin described by Thomsen (21), and more completely characterized by Friedenreich (27). This phenomenon was initially induced by the enzymic activity of certain bacteria which rendered erythrocytes panagglutinable by normal serums. It was postulated that the pan-agglutinability was attributable to the exposure of the "T" antigen on the erythrocyte surface. As stated previously, other treatments of erythrocytes may result in antigenic alterations of the erythrocyte surface, although not necessarily by the same mechanism.

The antibodies in some normal serums with specificities to various of these modified erythrocytes probably are produced subsequent to development of analogous or identical antigenic sites on the host's erythrocyte during the natural degradation process (26). Certain of these panagglutinins also have been implicated in several pathologic states (28–32), and may represent a response to subtle changes in the patient's physiology, referable to the erythrocyte.

From the data here, it is apparent that the two types of erythrocyte treatment each imparted a commonly shared antigenic specificity to the test erythrocytes, as shown by the reciprocal adsorption experiments. In addition to this commonly held specificity, each erythrocyte possesses a more predominant antigenic capacity as measured by reactivity with its respective antibody(ies).

The failure of the normal rabbit serums to react directly with OT-treated sheep erythrocytes does not preclude the presence of antibodies to the "common antigen" on this erythrocyte, since homologous adsorptions of these serums also removed the antibodies to the trypsinized erythrocyte.

Investigations are being contemplated at present to extend the observations herein described. It will be of interest to examine serums from tuberculous patients for the possible presence of antibodies to the trypsinized erythro-

cyte. Studies are also planned to ascertain the significance of the antibodies to OT-modified erythrocytes frequently encountered in nontuberculous persons, and with sufficient incidence in some studies so as to invalidate this procedure as a serodiagnostic implement. It is possible that such reactions represent antibodies to the commonly shared pan-agglutinogen and could be removed from consideration by adsorption.

Summary

Antibodies are produced to erythrocyte-adsorbable components of Old Tuberculin (OT) by the injection of rabbits with treated sheep or rabbit erythrocytes. Although the specificities of these two erythrocyte antigens are similar, as shown by cross-adsorption studies, the antibody produced in response to each varies in rate and extent. This phenomenon may be attributable to a stimulatory effect of the erythrocyte "carrier." The trypsinized erythrocyte reacted to low titers with all normal and immune serums tested, while the OT-sensitized sheep erythrocyte reacted only with the immune systems. The results of cross-adsorption studies suggest an antigenic specificity held in common by these two types of modified erythrocytes.

Sumario

Relación Serológica de los Eritrocitos Ovinos Sensibilizados con Tuberculina Antigua y los Eritrocitos Humanos Tripsinizados

Se producen anticuerpos a los componentes de la tuberculina antigua (TA) adsorbibles por los eritrocitos por la inyección en conejos de eritrocitos tratados de oveja o de conejo. Aunque son semejantes las especificidades de estos dos antígenos eritrocíticos según revelan los estudios de adsorción cruzada, el anticuerpo producido en respuesta a cada uno varía en su proporción y capacidad. Cabe atribuir este fenómeno al efecto excitante del "schlepper" eritrocítico. El eritrocito tripsinizado reaccionó a títulos bajos a todos los sueros normales e inmunes ensayados, en tanto que el eritrocito ovino sensibilizado con TA no reaccionó más que con los inmunes. Los resultados de los estudios de adsorción cruzada sugieren una especificidad antigénica poseída en común por estas formas de eritrocitos modificados.

Resume

Relation sérologique entre les érythrocytes de mouton sensibilisés à la tuberculine de Koch et les érythrocytes humains traités par la trypsine

Des anticorps aux composants érythrocyto-adsorbables de la tuberculine de Koch (OT) sont produits par l'injection au lapin d'érythrocytes de lapin ou de mouton traités. Bien que les spécificités de ces deux antigènes érythrocytaires soient semblables, comme l'indiquent les études sur l'adsorption croisée, la vitesse et l'importance de la réaction anticorps varient pour chacun d'eux. Ce phénomène serait imputable à un effet de stimulation sur l'érythrocyte "schlepper". L'érythrocyte trypsinisé réagissait à faible dosage avec tous les sérums normaux ou immuns testés, alors que l'érythrocyte de mouton sensibilisé à la tuberculine de Koch ne réagissait qu'avec les systèmes immuns. Les résultats des études sur l'adsorption croisée suggèrent l'existence d'une spécificité antigénique appartenant en commun à ces deux types d'érythrocytes modifiés.

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ANTIGENICITY OF ANTIMYCOBACTERIAL SERUMS[1,2]

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INTRODUCTION

The presence of inhibitors was shown by Gerstl and his associates (1, 2) to be responsible for the serologic nonreactivity of certain tuberculous serums when tested with Old Tuberculin (OT)-sensitized erythrocytes. While serologic reactivity was restored to one or more of the fractions obtained by ethanol treatment of such serums, the identity of the inhibitor, however, was not established.

In this same regard, Seibert and associates (3, 4), during the course of investigating experimental tuberculous disease in rabbits and guinea pigs, suggested that serum polysaccharides, resembling the bacillary carbohydrate, may interfere with *in vitro* antibody demonstrations.

The results of the previous study (5) suggested a method by which antigenic polysaccharides, possibly contained in antimycobacterial serums, might be demonstrated. Selected antiserums from rabbits injected with mycobacteria were incorporated in adjuvant, free of tubercle bacilli, and introduced into normal animals. These antiserums, in turn, were examined for reactivity with OT-sensitized sheep erythrocytes, with trypsinized human erythrocytes, and with both modified test erythrocytes after adsorption procedures.

MATERIALS AND METHODS

Techniques for the modification of the erythrocytes, collection and treatment of serums, serologic tests, and adsorptions were performed as described in the previous report (5).

Immunization of animals: Young adult rabbits weighing 2 to 3 kg. each were employed for all immunization procedures. Antimycobacterial serums were obtained following intravenous injection of 0.02 mg. of viable *Mycobacterium tuberculosis* var. *bovis* (Ravenel), or 1.0 mg. of either viable or nonviable suspensions of the other mycobacterial species as indicated in the text. All species had been cultivated on Löwenstein medium at 37°C. until adequate growth was observed and were then harvested into weighing bottles. The nonviable inocula were autoclaved subsequent to weighing, and saline (phosphate-buffered to pH 7.4) was added to all samples to effect the desired weight of inoculum per ml.

One-milliliter aliquots of the serums, collected at weekly intervals from the above animals, were incorporated into a modified Freund's adjuvant and injected intramuscularly into normal rabbits. This adjuvant differed from the usual Freund's adjuvant in that it was free of mycobacteria. The serum aliquot was mixed with 1.0 ml. of melted falba, followed by the addition of 2.0 ml. of mineral oil. Thorough mixing was accomplished by repeatedly filling and expelling the preparation from a tuberculin syringe, while maintaining the temperature of the ingredients at 56°C. Tubes containing the adjuvant mixture were observed for a period of five to ten minutes before the addition of the mineral oil to assure the proper emulsification of the water and oil phases.

Aliquots of all serums to be incorporated into adjuvant were previously examined for mycobacteria by culture on Löwenstein medium. This sterility testing was performed twice. In no instance was there evidence of mycobacterial growth.

EXPERIMENTAL RESULTS

Response of rabbits to injections of antimycobacterial serums:

To control the influence of adjuvant alone on the serologic response of rabbits to the two types of modified erythrocyte test antigens, a 4.0-ml. aliquot of the falba and mineral oil adjuvant (*see* Materials and Methods) containing saline, in lieu of serum, was injected intramuscularly into normal rabbits. Weekly serum samples were then tested with OT-sensitized sheep erythrocytes and with trypsinized human erythrocytes.

As shown in figure 1, hemagglutinins to both test antigens were measurable, but in low titers. The results of adsorption studies revealed that it was possible to remove all antibody with either antigen. On the basis of the preceding report (5), this mutual adsorbability might serve to establish the presence of specific antibodies in tuberculous serums inasmuch as these are not completely removed by the adsorption with the trypsinized human erythrocyte.

[1] From the Department of Bacteriology, Ohio State University, Columbus 10, Ohio.

[2] This study was supported in part by a grant from the Ohio Tuberculosis and Health Association.

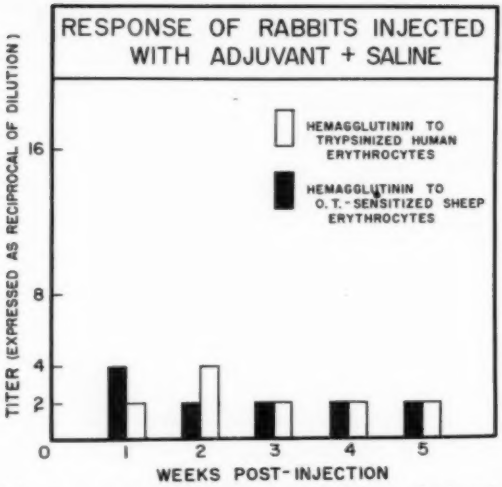

FIG. 1. Hemagglutinin response of rabbits injected with a falba and mineral oil adjuvant alone.

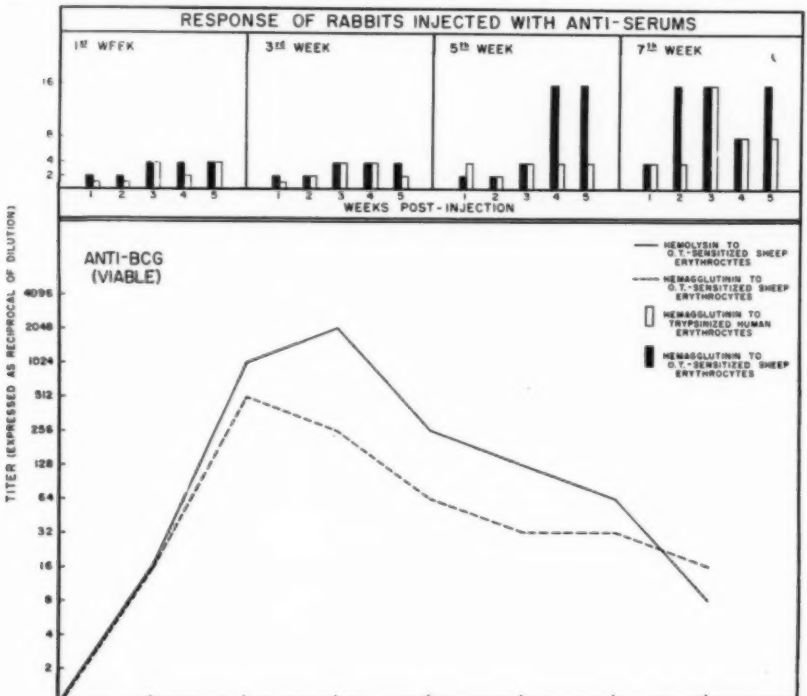

FIG. 2. Antigenicity of anti-BCG serums as demonstrated by the production of hemagglutinins to OT-sensitized sheep erythrocytes.

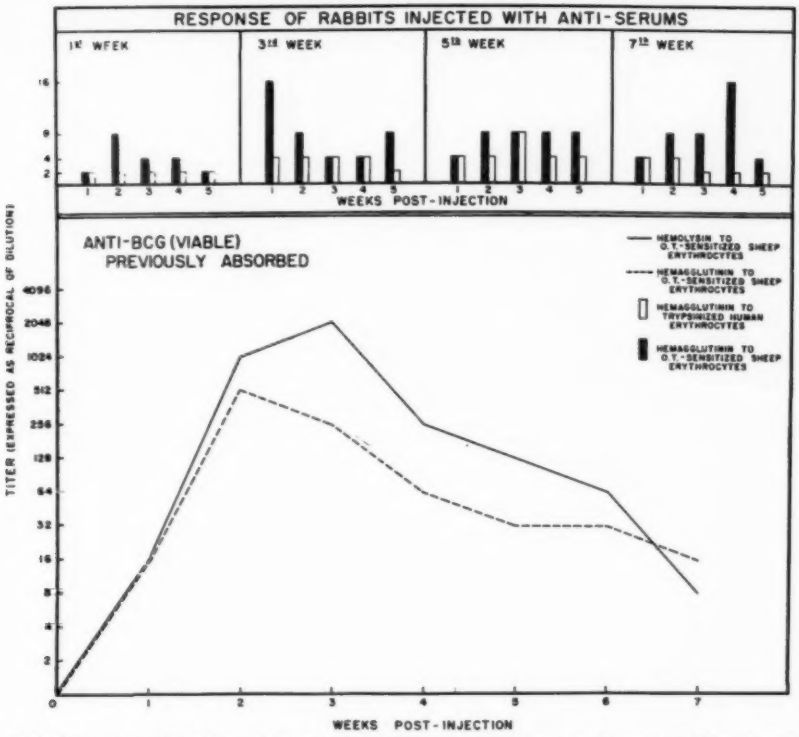

FIG. 3. Influence of prior adsorption to OT-sensitized erythrocytes on the antigenicity of anti-BCG serums. Compare with figure 2.

Subsequently, selected serums from animals injected with viable *M. tuberculosis* var. *bovis* (Ravenel) and with BCG were incorporated in adjuvant and introduced into normal rabbits by the intramuscular route. Antiserums, recovered from blood samples procured at weekly intervals from these rabbits, were properly inactivated, adsorbed, and titrated with both OT-sensitized sheep erythrocytes and trypsinized human erythrocytes.

The antibody curves in figure 2 are representative of the hemagglutinin and the hemolytic antibody response following the injection of BCG. Injection of selected weekly serums from this animal, including the pretreatment sample, produced the serologic responses illustrated by the bar graph. Titers of the hemagglutinins to the OT-treated sheep and the enzyme-modified erythrocytes are indicated by the height of the respective bars. For purposes of illustration, the bars are represented as an arithmetic projection in contrast to the graph which is a geometric projection. Although the hemolytic modification of the Middlebrook-Dubos test was performed, no positive demonstrations were observed with this particular system.

The injection of pretreatment or baseline serums always resulted in serum titers similar to those observed to the adjuvant alone and, consequently, are not represented in the individual graphs. The results of injecting first- and third-week antiserums resembled those noted after the injection of normal serum, with hemagglutinins present only in the lower dilutions (1:2, 1:4) when tested with each of the modified erythrocytes. An increase in anti-OT titers to levels above those seen following the injection of normal serum or adjuvant alone occurred in animals which had received the fifth- or seventh-week antiserums. This increase in titer paralleled the diminution of circulating antibody in the im-

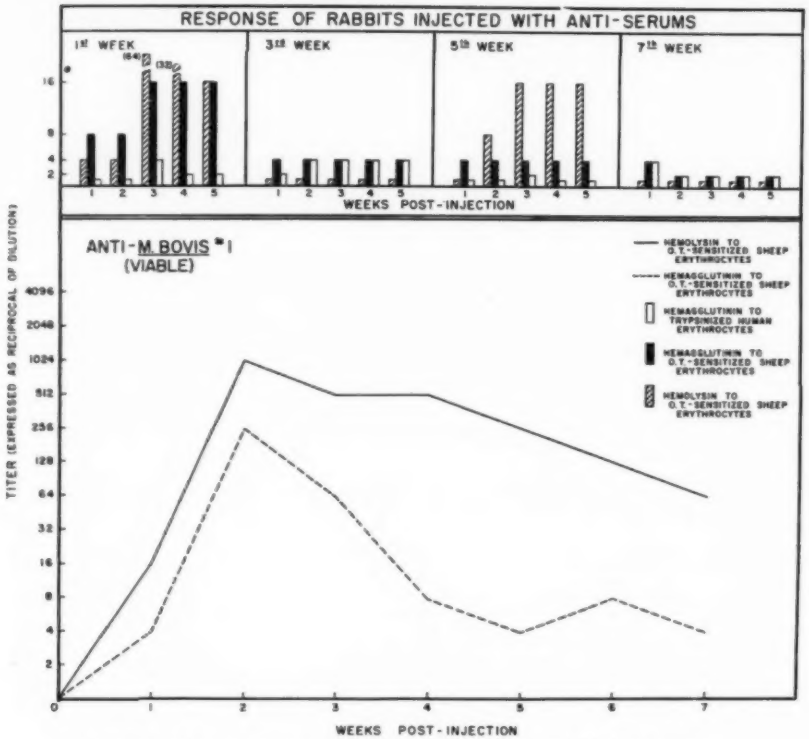

FIG. 4. Serologic response of rabbits injected with anti-*M. bovis* serums.

mune serums, as indicated by the declining slopes of the antibody curves.

It should also be noted that the titers of the hemagglutinins directed to the human trypsinized erythrocyte approximated those observed in normal serums, except for the serologic response to the injection of the seventh-week antiserum. This is the only instance in which this occurred during the current study. However, the existence of similar agglutinin titers to this enzyme-treated cell has been reported with certain other immune systems, not too dissimilar from the OT-anti-OT-system (5), and these titers were readily removed by adsorption with OT-modified sheep erythrocytes. The exaggerated antibody response to this particular serum may be the result of a polysaccharide peculiar to it.

When these same anti-BCG serums were completely adsorbed with OT-sensitized sheep erythrocytes prior to being injected into normal rabbits, the serologic response varied from that described above. The antibody titers to the OT-sensitized erythrocyte in serums from the rabbit injected with the third-week antiserum were significantly increased over those demonstrated in normal serum (figure 3). Titers of hemagglutinins to the trypsinized human erythrocyte again approximated those associated with the control serums and remained at low levels even in the serums from the animal which had received the seventh-week antiserum. This was in contrast to the results obtained from injecting the same serum before adsorption with the OT-treated erythrocytes.

Identical experiments were conducted with the antiserums from animals which had been injected with *M. tuberculosis* var. *bovis* (Ravenel), the results of which are graphically illustrated in figure 4. The curves representing antibody pro-

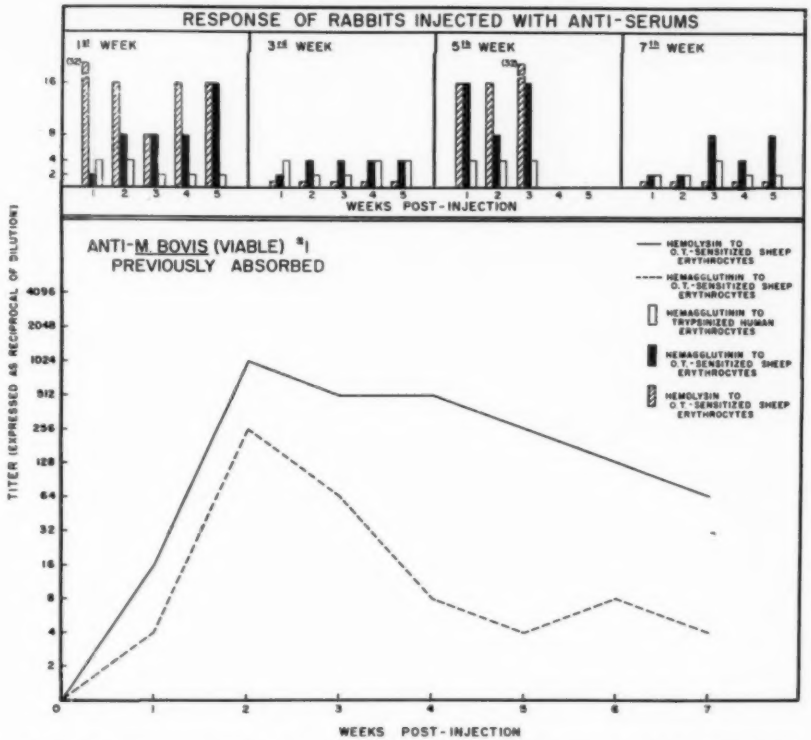

FIG. 5. Antibody response of rabbits injected with anti-*M. bovis* serums which had been previously adsorbed with OT-treated sheep erythrocytes. Compare with figure 4.

duction to the bacilli resembled the response observed following the injection of BCG; however, the injections of these immune serums resulted in demonstrations different from those described for the anti-BCG serums. Apparently the first- and fifth-week antiserums contained hemagglutinogens and lytic antigens since the corresponding serums gave both types of demonstration. The later antiserums were devoid of these antigens. It should be noted that hemolysin titers greater than 1:16 are indicated by the dilution. As before, antibodies to the enzyme-treated erythrocyte were demonstrated but to a low level.

Adsorption of these antiserums before injection did not appreciably affect the serologic response except that the maximal titers were achieved sooner after inoculation (figure 5). This agrees substantially with the results obtained by the injection of the adsorbed anti-BCG serums.

These experiments were duplicated with similar results. However, the titers obtained from injecting antiserums from a third rabbit which had been infected with *M. bovis* presented a different picture (figure 6). The initial response to the organism differed from that of the first rabbit in that maximal antibody titers were not observed until later in the course of infection, shortly after which the animal died, and the presence of antigen in these antiserums could not be demonstrated by the testing procedures employed. At present, this is accounted for only by the individual variation in the response of test animals.

Cross-adsorption studies of antiserums to tuberculous serums: In the previous study (5), it was shown that anti-OT substances (other than those to the mycobacteria or their products) present in antiserums are adsorbable with the trypsinized

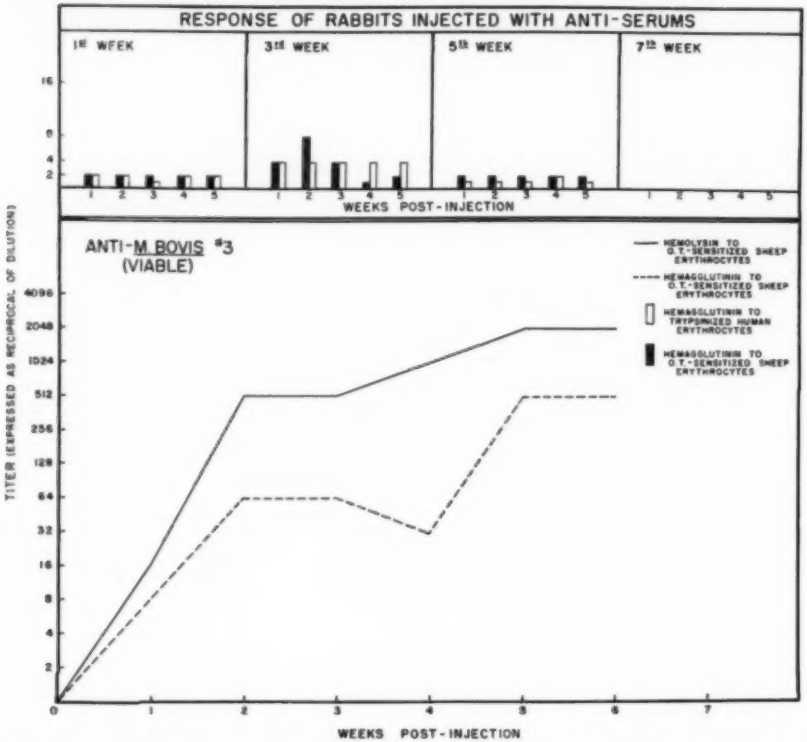

FIG. 6. Antigenicity of anti-*M. bovis* serums from a rabbit whose initial serologic response differed from that of the animal illustrated in figure 4.

human erythrocytes. As mentioned earlier in the present report, the injection of adjuvant alone elicited an antibody response to OT-sensitized sheep erythrocytes which also was adsorbable with the enzyme-treated erythrocyte. In order to investigate the specificity of the response demonstrated by the injection of the antimycobacterial serums, similar adsorption techniques were employed.

The results of reciprocal adsorptions are represented in table 1, the two types of modified erythrocytes serving as the adsorbents of the serums graphically illustrated in figure 2 (anti-BCG).

As indicated, adsorption with the enzyme-modified cell removed all of the antibodies to OT-sensitized sheep erythrocytes present in serums of animals injected with the earlier anti-BCG serums, leaving only those antibodies specifically directed to antigens present in OT. Apparently specific antigenic substances were also present in the third-week anti-BCG serum as well as in the fifth- and seventh-week serums. These results are in accord with those obtained by the adsorption of the third-week immune serum with OT-sensitized sheep erythrocytes prior to its injection into a normal rabbit (*see* figure 3). The adsorption of these serums with the OT-treated cell, on the other hand, not only removed all specific substances, but also those directed to the trypsinized erythrocyte, which supports the data presented previously for other immune systems (5).

Similar results (table 2) were obtained upon testing the serum of rabbits injected with the anti-*M. bovis* serums (*see* figure 4). Here again adsorption with trypsinized erythrocytes removed all antibodies considered to be common

TABLE 1

INFLUENCE OF RECIPROCAL ADSORPTIONS ON THE ANTIBODY DEMONSTRATIONS OF RABBITS INJECTED WITH ANTI-BCG SERUMS

Test Antigen	Weeks post Injection	Serologic Response to Injection of Anti-BCG (Viable) Serums			
		Weekly Bleeding Injected (Serum) First	Third	Fifth	Seventh
OT-sensitized sheep erythrocytes	1	2	2	2	4
	2	2	2	2	16
	3	4	4	4	16
	4	4	4	16	8
	5	4	4	16	16
	1	—	—	4	8 *
	2	—	—	4	8
	3	—	—	16	8
	4	—	8	8	16
	5	—	4	8	16
Trypsinized human erythrocytes	1	+†	+	4	4
	2	+	2	2	4
	3	4	4	4	16
	4	2	4	4	8
	5	4	2	4	8
	1	—	—	—	—
	2	—	—	—	—
	3	—	—	—	—
	4	—	—	—	—
	5	—	—	—	—

* [ ] Indicates heterologous adsorption.
† Reactive only as undiluted serum.

to both treated erythrocyte test antigens, leaving hemagglutinins specifically directed to antigens of OT. These were found only in the serums of those animals which had been injected with the first- and fifth-week anti-*M. bovis* serums. Specific hemolysins, however, were adsorbable with the enzyme-treated cell, although the titers obtained originally were substantially higher than were those for the hemagglutinins.

This is in keeping with the previous observation (5) that antibodies may possess different avidities for the trypsinized erythrocyte, which in turn may reflect differences in their specificities.

Serologic response and adsorption studies of rabbit serums prepared by the injection of nonviable and saprophytic mycobacteria: Evidence has been presented which suggests that specific antigenic substances in some form are present in certain tuberculous antiserums. Identical experiments to those just described were conducted with nonviable *M. tuberculosis* var. *bovis* (Ravenel), *M. tuberculosis* var. *hominis*, and BCG, as well as viable and nonviable *M. phlei*. The initial antibody curves were not too dissimilar from those recorded for the viable organisms except for the magnitude of titers. Injection of selected antiserums from these animals failed to present evidence, by the tests employed, of any antigen in the serums introduced. The antitrypsinized human erythrocyte titers also approximates those obtained before. In addition, cross-adsorption studies resulted in complete removal of all anti-substances with either treated cell antigen.

DISCUSSION

It appears from the data presented here and in the preceding paper (5) that at least two specific antigenic components (or groups of components) of Old Tuberculin are adsorbable to the surface of sheep erythrocytes. One of these antigens possesses a specificity similar to, or identical with, a specificity exhibited by the trypsinized human erythrocyte, and antibodies directed to this determinative group can be removed from rabbit serum by adsorption with either treated cell.

Although normal rabbit serums contain antibodies measurable with the enzyme-modified erythrocyte, none is usually demonstrable with the OT-sensitized erythrocyte. Upon immunization with a variety of unrelated antigens (5) or with adjuvant free of tubercle bacilli and containing normal serum, antibodies to the tuberculin-treated erythrocyte appear; but these, like the antibodies in normal serum to the trypsinized erythrocyte, are in low titer and for the most part may be removed by homologous or heterologous adsorption. In this regard, Dray and Young (6) were able to demonstrate the presence of isopreciptinogens in rabbit serums by injecting normal serums with adjuvant into the homologous species, and it is possible that the response to normal serums, as measured with the OT-sensitized sheep erythrocyte, may be due to this type of stimulus.

Thus, it would appear that these antibodies, measurable with both modified cells, are naturally occurring substances or may be stimulated readily by immunization. The frequency with which they are observed has suggested the

TABLE 2

INFLUENCE OF RECIPROCAL ADSORPTIONS ON THE ANTIBODY DEMONSTRATIONS OF RABBITS INJECTED WITH ANTI-M. BOVIS SERUMS

Test Antigen	Weeks post Injection	Serologic Response to Injection of Anti-*M. bovis* (Viable) Serums							
		Weekly Bleeding Injected (Serum)							
		First		Third		Fifth		Seventh	
		HL*	HA	HL	HA	HL	HA	HL	HA
OT-sensitized sheep erythrocytes	1	4	8	—	4	—	4	—	4
	2	4	8	—	4	8	4	—	2
	3	64	16	—	4	16	4	—	2
	4	32	16	—	4	16	4	—	2
	5	16	16	—	4	16	4	—	2
	1	—	4	—	—	—	—	—	— †
	2	—	4	—	—	—	2	—	—
	3	—	16	—	—	—	4	—	—
	4	—	8	—	—	—	2	—	—
	5	—	8	—	—	—	2	—	—
Trypsinized human erythrocytes	1	—	+‡	—	2	—	+	—	4
	2	—	+	—	4	—	+	—	2
	3	—	4	—	4	—	2	—	2
	4	—	2	—	4	—	+	—	2
	5	—	2	—	4	—	2	—	2
	1	—	—	—	—	—	—	—	—
	2	—	—	—	—	—	—	—	—
	3	—	—	—	—	—	—	—	—
	4	—	—	—	—	—	—	—	—
	5	—	—	—	—	—	—	—	—

* HL refers to hemolysis; HA to hemagglutination.
† [ ] Indicates heterologous adsorption.
‡ Reactive only as undiluted serum.

terms "common antibody" and, for the corresponding antigens, "common antigen," but only for purposes of this discussion.

In addition to the common antigen, the OT-sensitized erythrocyte demonstrates a specificity for antibodies present in antimycobacterial serums. That these antibodies are distinct from the common antibodies was established by their titer and nonadsorbability with the trypsinized human erythrocyte.

By injecting certain antimycobacterial rabbit serums into the same species, it was possible to stimulate the production of antibodies specific for OT-sensitized erythrocytes. These antibodies, although generally in low titers, were not removed by adsorption with the enzyme-modified human erythrocyte and, demonstration-wise, were identical with the antibodies present in the original antimycobacterial serums.

The only serums possessing this antigenicity were from rabbits which had received viable *M. bovis* or BCG. Serums from rabbits injected with nonviable suspensions of these organisms or with saprophytic species were apparently devoid of this peculiar capacity and behaved like normal serum, as evidenced by testing the corresponding antiserums.

Adsorption of the antigenic serums with OT-sensitized erythrocytes prior to their injection into normal rabbits, so as to remove all demonstrable antibodies, did not impair their antigenicity. Thus, it seems improbable that the antigen in question is bound firmly, if at all, to the antibody removed with the OT-treated cell, although elution from antibody fixed to the erythrocyte exists as a possibility. It is presumed that this antigen is carbohydrate or contains carbohydrate, so as to liken it to the erythrocyte-adsorbable antigens in Old Tuberculin. The failure to sensitize susceptible erythrocytes with

these antigenic serums (experiments not reported here) is not surprising, since it has been reported by Rheins and Thurston (7) that serum, specifically serum albumin, interferes with the adsorbability of antigens in OT. This statement assumes the existence of the serum antigen as a free entity. In fact, it may well be present as a soluble antigen-antibody complex, a prosthetic group of a serum protein or, for that matter, in all forms. Should it somehow combine with antibody *in vivo*, the resultant antibody depression might account for the results described by Gerstl and his co-workers (1, 2), who successfully converted serologically nonreactive tuberculous serums to seroreactivity by alcohol fractionation. The procedures employed may have split antigen-antibody complexes, permitting the subsequent demonstration of antibody.

In this connection, it might be possible by the adsorption techniques, described here and in the foregoing paper (5), to establish the significance of the so-called false-positive reactions observed with certain nontuberculous serums when tested with OT-sensitized erythrocytes.

Seibert and her associates (8–10) described changes in the content of serum polysaccharide in tuberculosis and other chronic diseases characterized by tissue degradation, and assigned to this component the electrophoretic mobility of alpha-2 globulin. Although not identifiable as tuberculopolysaccharide in tuberculosis, Seibert and associates (3) have wondered whether in certain situations free tuberculopolysaccharide might interfere with the development of immunity, at least in the experimental disease.

In the present study no effort was made to associate the serum antigen with tuberculopolysaccharide. Since its presence was ascertained only in those serums from rabbits injected with viable mycobacteria known to be capable of becoming established in tissue (*M. bovis* and BCG), it may represent a product (or products) of tissue degradation. Should this prove to be the case, it is obvious that this substance then is quite similar to, and perhaps identical with, a component present in OT which is adsorbable to the sheep erythrocyte. While it is rather difficult to stimulate the production of antituberculopolysaccharide with other than the most purified antigens or with polysaccharides in the haptenic form (11), the demonstration of the serum antigen in these studies was accomplished readily by means of adjuvant. This may be interpreted as indicating that the antigen is not tuberculopolysaccharide but a tissue product, or that it is tuberculopolysaccharide conjugated to protein.

At present, these studies are being extended and will include attempts to elicit skin reactions in sensitized animals by injecting antigenic serums, as well as examinations of these serums, to determine the character of these antigenic substances.

Summary

Injection of adjuvant or normal serum in adjuvant stimulated the production of serum antibodies in rabbits to Old Tuberculin (OT)-treated erythrocytes. These substances are removed by adsorption with either the OT-modified erythrocyte or with the trypsinized human erythrocyte which strongly suggests a common antigenic component for both types of modified erythrocyte.

Rabbits receiving antiserums from animals injected with culturable *Mycobacterium* var. *bovis* or with BCG elicited a heightened response to the OT-sensitized erythrocyte which could not be removed by adsorption with the enzyme-modified human erythrocyte. The antigenicity of these serums was unaltered by adsorption with OT-sensitized sheep erythrocytes prior to injection into normal rabbits. These results indicate that these serums contain antigenic substances, presumably polysaccharides, which are identical with, or similar to, erythrocyte-adsorbable components in OT.

Similar procedures employing serums from animals injected with nonviable and saprophytic species of mycobacteria produced results comparable to the injection of normal serums.

Sumario

Antigenicidad de los Sueros antimicobacterianos

La inyección de un coadyuvante o de suero normal en un coadyuvante excitó en los conejos la producción de anticuerpos séricos para los eritrocitos tratados con tuberculina antigua (TA). Estas substancias se eliminan por la adsorción ya con eritrocitos modificados por la TA o con eritrocitos humanos tripsinizados, lo cual sugiere poderosamente la existencia de un componente antigénico en común para ambas formas de eritrocitos modificados.

Los conejos que recibieron antisueros de los animales inyectados con *Mycobacteria bovis* o con BCG mostraron una respuesta acentuada al

eritrocito sensibilizado por TA, que no podía eliminar la adsorción con el eritrocito humano modificado por la enzima. La antigenicidad de estos sueros no fué alterada por la adsorción con eritrocitos ovinos sensibilizados con TA antes de inyectarlos en conejos normales. Indican tales resultados que estos sueros contienen substancias antigénicas, presuntamente polisacáridos, que son idénticas o semejantes a los componentes de la TA adsorbibles en los eritrocitos.

Procedimientos semejantes en que se emplearon sueros de animales inoculados con especies aviables y saprofitas de micobacterias produjeron resultados comparables a los de la inyección de sueros normales.

Resume

Antigénicité des sérums antimycobactériens

L'injection de l'adjuvant ou de sérum normal dans l'adjuvant stimulait la production d'anticorps sériques chez des lapins dont les érythrocytes avaient été traités par la tuberculine de Koch (OT). Ces substances sont extraites par adsorption sur des érythrocytes modifiés par la tuberculine de Koch ou des érythrocytes humains trypsinisés, ce qui suggère fortement l'existence d'un composant antigénique commun pour les deux types d'érythrocytes modifiés.

Les lapins recevant l'antisérum provenant d'animaux injectés avec le *Mycobacterium bovis* ou avec le BCG ont présenté une réaction renforcée à l'érythrocyte sensibilisé par la tuberculine de Koch, la réaction ne pouvait être supprimée par adsorption sur l'érythrocyte humain modifié par l'enzyme. L'antigénicité de ces sérums n'était pas altérée par l'adsorption sur les érythrocytes de mouton sensibilisés par la tuberculine de Koch avant l'injection sur lapin normal. Ces résultats indiquent que ces sérums contiennent une substance antigénique, vraisemblablement des polysaccharides, qui sont identiques ou similaires aux composants érythrocyto-adsorbables de la tuberculine de Koch.

Des méthodes similaires utilisant les sérums d'animaux inoculés avec des espèces saprophytes non viables de mycobactéries a produit des résultats comparables à ceux de l'injection de sérums normaux.

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Case Reports

AGENESIS IN THE PULMONARY ARTERIAL CIRCULATION[1]

Report of Two Cases

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INTRODUCTION

As recently as 1952, Madoff, Gaensler, and Strieder (1) commented that congenital absence (agenesis) of a branch of the main pulmonary artery is a rare anomaly. They reported the first case diagnosed by clinical methods and were able to find only nine previously reported cases of this anomaly in the literature, all having been found at post-mortem examination or at surgery. Steinberg, Dotter, and Lukas (2) (1953) thought that, with the increasing use of angiocardiography, more cases in this category would be found. This has proved to be true.

It is believed that consideration of this category of patients should not be limited to those patients in whom there is total absence of either the right or the left branch of the main pulmonary artery, but should be extended to include those in whom there is a developmental defect (agenesis) of a major portion of either or both of these branches. This does not include those patients in whom there has been an abnormal development of the lung parenchyma with a resultant defect in the vasculature of that lung.

There are two reasons for inclusion of this type of defect. First, the therapeutic considerations, based on the physiologic factors involved, are the same whether the entire lung has an abnormal circulation or only a major portion of the lung is involved. Second, the embryologic basis for either defect, if not the same, is certainly related.

If this inclusion is accepted, the earliest case report for such a defect is credited to Maugar (1802) by Findlay and Maier (3). There is recorded in the literature a total of fifty-one cases (1–21) of agenesis of a branch of the main pulmonary artery, two additional cases being reported in the present study. This is not an accurate figure since several of the records are scanty, and considerable doubt exists whether the primary defect is actually in the pulmonary arterial circulation. Sweet and White's case (10), for example, is included only for historical reasons since it was, in fact, a persistent truncus. The same is true for those cases of Danelius (7) and Manhoff and Howe (9). The second case in the paper by Findlay and Maier (3) was apparently a partial agenesis of the lung with a concomitant defect in development of the pulmonary artery. While there is no certainty, it is probable that others of the earlier cases do not represent true agenesis of a branch of the pulmonary artery. Further evaluation of this anomaly will depend upon complete documentation to include a complete cardiopulmonary history; physical findings; an electrocardiogram; conventional chest films in oblique and lateral, as well as postero-anterior, projections; laminagrams; bronchograms; angiocardiograms; bronchospirometric studies; and tissue sections, when available.

Unfortunately, the recent increase in case finding has not been accompanied by a corresponding improvement in an understanding of the embryologic factors which govern the form and severity of the anomalies encountered. Investigators are generally in agreement with regard to the very early stages of development of the pulmonary circulation, but there is a divergence of opinion regarding the later stages, and no single concept can explain all of the variations. The anomalies are not usually confined to the pulmonary artery or its branches, but frequently include changes in the heart or in the systemic circulation. To complicate matters further, the altered physiology is additionally responsible for some of the changes seen. Maier (16) observed that it was logical to assume that the earlier the embryologic aberration occurs in one component of the dual circulation, the more extensive the changes in the other will be. In addition, Manhoff and Howe (9) noted that the anomaly that devel-

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ops depends upon the stage at which development becomes abnormal.

There is not, as yet, a sufficient number of clearly documented cases to warrant statistical generalizations regarding sex incidence or side preference for the defect to occur. However, it is apparent that agenesis in the pulmonary arterial circulation is less frequently found as the only defect than when it is accompanied by congenital anomalies of the heart and other major vessels. In addition, Nadas and associates (13) attach significance to the combination of absence of the *left* pulmonary artery and Fallot's tetralogy. This has been more extensively covered by Emanuel and Pattinson (20). The practical significance of this latter point, while not always constant, is that absence of the *left* pulmonary artery is usually associated with Fallot's tetralogy, while absence of the *right* pulmonary artery is found either with a normal heart or a congenital lesion confined to the great vessels.

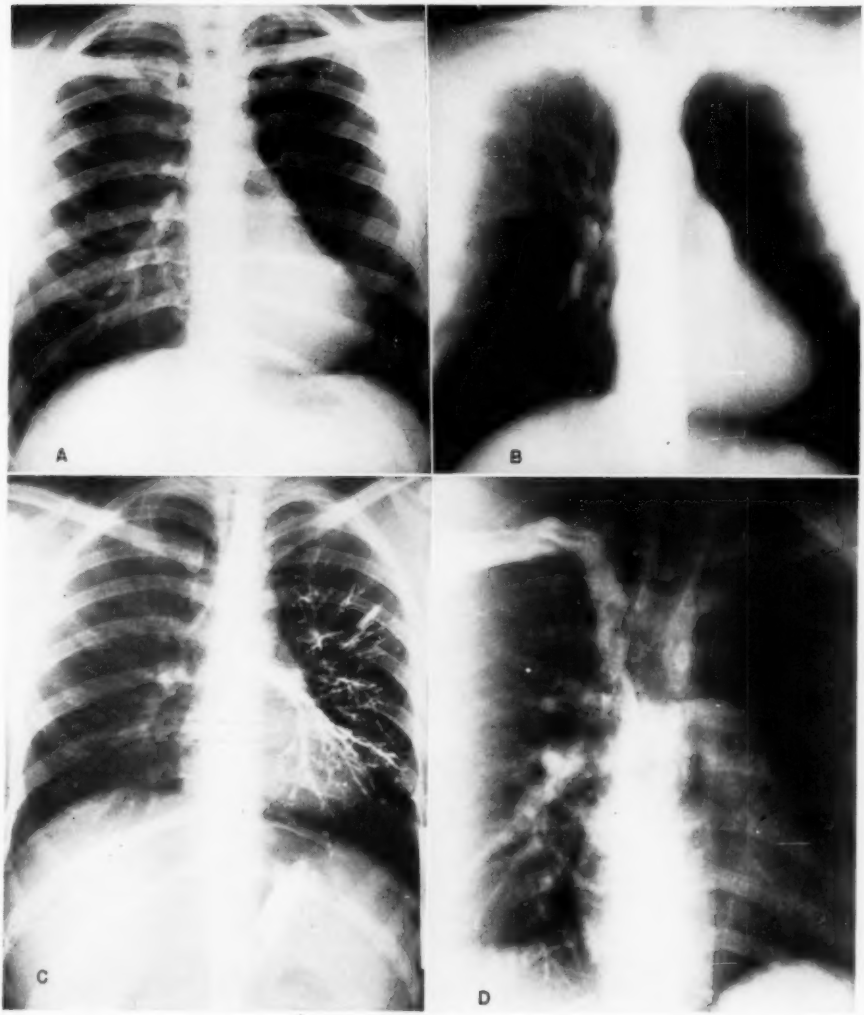
Another anatomic finding in agenesis of the pulmonary artery which is of practical importance is related to collateral circulation. When the absent pulmonary artery is not associated with other congenital defects, hypertrophy and dilatation of the bronchial arteries occur. Bloomer and his co-workers (22) have shown this experimentally, and Bing and his associates (23) have shown that in most cases of pulmonary stenosis the pulmonary capillary flow exceeded the pulmonary arterial flow, demonstrating the presence of collateral circulation to the lung. Many of the case reports on absence of the pulmonary artery indicate a frequent incidence of aberrant arterial supply to the affected lung. These aberrant vessels and the bronchial arterial supply may be difficult to demonstrate by angiocardiography, but the presence of one or the other abnormality is almost assured and must be searched for in any patient for whom surgery is contemplated. It will be noted that the patients of both cases reported here showed cystic lesions also in the affected lung. Bruwer and associates (24) and Findlay and Maier (3) have noted that anomalous systemic arteries to lungs are not an unusual finding in association with bronchogenic cysts, congenital bronchiectasis, and sequestration. They are a less common finding when there is no evidence of gross anomalous development of pulmonary tissue.

Case Reports

Case 1. A 23-year-old Syrian male was referred to Fitzsimons Army Hospital in February, 1957, because of chest pain, shortness of breath, and an abnormal chest roentgenogram. He stated that, as a child, he was free of upper respiratory infections, pneumonias, or shortness of breath, and was able to participate effectively in athletics. He immigrated to the United States in 1951, following which he had recurrent upper respiratory infections, but no persistent cough between infections. He first noted shortness of breath on exertion with associated vague, substernal pain, when he was inducted into military service six months before admission to the hospital. He found that he was unable to march at double-time with the rest of his unit without stopping to rest.

Physical examination: Physical examination on admission revealed: temperature, 98.6°F.; pulse, 72 per minute; respirations, 20 per minute; blood pressure, 120 systolic, 80 diastolic in mm. of mercury. The patient was an asthenic male who was in no distress. The chest was symmetrical, with bilaterally equal and adequate excursions. A few scattered fine and medium inspiratory rales were heard over the left lower lung field. A post-tussic maneuver was normal. Examination of the cardiovascular system failed to reveal any murmur, arrhythmia, cardiomegaly, or discrepancy in pulses. The aortic second sound was greater than the pulmonic second sound, with a split-second sound over the pulmonary area. The remainder of the physical examination was normal.

Laboratory data: The urinalysis and the cardiolipin microflocculation test were negative. The leukocyte count was 8,700 per cu. mm. with a normal differential cell count; the hemoglobin was 19.2 gm.; hematocrit, 53; and the sedimentation rate, 5 mm. per hour. The tuberculin skin reaction to second-strength PPD was 2-plus; the histoplasmin and coccidioidin reactions were negative. The electrocardiogram was normal. The chest roentgenogram (figure 1*A*) showed a slight mediastinal shift to the left, increased radiolucency of the entire left lung field, and emphysematous blebs at the left base. The contour of the heart appeared to be within normal limits, but there was a suggestive decrease in the size of the left pulmonary outflow tract. A laminagram (figure 1*B*) showed some narrowing of the left pulmonary artery just proximal to arborization which was considered typical of anomaly of the left pulmonary artery. Bronchography (figure 1*C*) disclosed that a normal number of bronchopulmonary segments were present. Mild cylindrical bronchiectasis was demonstrated in the basilar segment of the left lower lobe and in the anteroposterior segment, left upper lobe. Angiographic studies (figure 1*D*) showed a normal right heart. The bifurcation of the pulmonary artery was normally situated, and the entire vascular tree on the right appeared to be normal. The superior division of the left pulmonary artery was present, but was considerably



Case 1

FIG. 1A. Postero-anterior roentgenogram showing slight mediastinal shift to the left, increased radiolucency of the left lung, and decreased size of the left pulmonary outflow tract.

B. Laminagram showing marked differences in the vascular shadows of each lung.

C. Left bronchogram demonstrating mild cylindrical bronchiectasis of the basilar segments, left lower lobe; and in the anteroposterior segment, left upper lobe.

D. Angiogram showing partial agenesis of the left pulmonary artery.

smaller than the comparable vessel on the right. This was the only visible pulmonary artery on the left side. The diagnosis was partial agenesis of the left pulmonary artery, particularly lower lobe ramification.

Course in the hospital: The patient was relatively asymptomatic except for mild exertional dyspnea when admitted to the hospital. He was afebrile, had no cough, and was ambulatory. Bronchoscopy showed gross variation in the position of the left

TABLE 1
PULMONARY FUNCTION STUDIES

External Spirometrics	Case 1		Case 2	
1. Vital capacity (cc.)				
Predicted	4,300		4,260	
Observed	3,380		3,360	
Per cent of predicted	89		86	
2. 1-second vital capacity				
Observed	2,780		2,550	
Per cent of predicted	73		70	
3. Maximal breathing capacity				
Predicted L/min.	131		140	
Observed	96.2		129	
Per cent of predicted	73		92	
Internal Spirometrics	**Right**	**Left**	**Right**	**Left**
4. Ventilation				
cc. (min.)	4,520	3,380	7,270	3,360
Per cent distribution	57	43	68	62
5. Oxygen consumption				
cc. (min.)	350	47	183	44
Per cent	88	12	81	19

basilar segmental bronchi. Fluoroscopic examination of the chest revealed impairment to the motion of the left diaphragm. Bronchospirometry studies (table 1) were compatible with incomplete agenesis of the left pulmonary artery. The patient was separated from the service.

Case 2. A 24-year-old Negro male was asymptomatic until January 16, 1957, when he noted the sudden onset of slight cough with hemoptysis of about one tablespoonful of bright red blood, associated with profuse diaphoresis and pleuritic pain in the left lateral and posterior chest. He was hospitalized and was treated with antimicrobial drugs for ten days, but showed no improvement. Chest roentgenograms showed some displacement of the cardiac silhouette to the left and decreased vascular markings in the left lung. On the basis of the symptoms and the roentgenographic abnormality, he was transferred to Fitzsimons Army Hospital for further evaluation.

Past medical history: In 1954, in Korea, the patient suffered a spider bite of the left hand followed by cellulitis requiring surgical drainage. At that time, he noted swelling in the left axillary region and was hospitalized. An axillary lymph node biopsy was interpreted as showing tuberculous lymphadenitis. The patient received antituberculous chemotherapy for thirteen months and was subsequently returned to duty.

Physical examination on admission revealed a temperature of 99°F.; pulse, 86 per minute; and blood pressure, 128 systolic, 80 diastolic in mm. of mercury. The patient appeared to be well developed, well nourished, and not chronically ill. The chest was symmetrical, with equality of expansion. The lung fields were normal except for decreased breath sounds in the left lateral chest, and in that area a few fine crepitant post-tussic rales were noted. Cardiovascular examination was negative except for a nontransmitted grade I, low-pitched, systolic murmur heard over the precordium. The aortic second sound was equal to the pulmonary second sound. No lymphadenopathy was present.

Laboratory studies: The urinalysis and cardiolipin microflocculation test were negative. The leukocyte count was 7,400 per cu. mm. with normal differential. Skin tests showed a 2-plus reaction to first-strength PPD, with negative coccidioidin and histoplasmin reactions. Blood-urea nitrogen, sickle cell preparation, and bromsulphalein and liver function studies were normal. Repeated cultures of sputum and gastric washings were negative for fungi and *M. tuberculosis.* Stool examinations for ova and parasites were negative.

An admission chest film (figure 2*A*) showed a partially calcified cystic shadow measuring 3 by 5 by 5.5 cm. in the extreme apex of the left lung. The left lung field was more radiolucent than the right. Vascular markings in the left lung field were minimal, and the left costophrenic angle was blunted. Laminagrams revealed the cystic lesion in the medial portion of the left apex previously described and confirmed the presence of calcium. The left pulmonary arterial shadow was not identifiable. A review of chest roentgenograms of May 1, 1953, and March 12, 1956, showed no change from current films. Left bronchograms (figure 2*B*) revealed terminal bronchiolar ectasia, particularly of the anterior segment of the left upper lobe. The right bronchogram was normal. Angiographic studies (figure 2*C*) showed good visualization of the right heart circulatory system with increased prominence of the pulmonary conus segment. The right pulmonary artery was definitely larger than normal, and only the inferior division of the left pulmonary artery was present. Ramifications of the inferior division of the pulmonary artery on the left were grossly normal in distribution but slightly reduced in size. The superior division of the left pulmonary artery was absent. Upper and lingular lobes appeared almost wholly supplied from anomalous sources. The electrocardiogram was suggestive of early left ventricular hypertrophy.

Course in hospital: During hospitalization, the patient had left chest pleuritic pain. Post-tussic crepitant rales in the left lateral lung field persisted.

Microscopic slides of axillary lymph node biopsy (1954) were reviewed, and granulomatous lymphadenitis was found. Prolonged study by three observers failed to reveal acid-fast bacilli. Pulmonary function studies (table 1) revealed a slight degree of ventilatory impairment of restrictive nature with a significant predominance in function to the right lung, both in ventilatory capacity

Case 2

FIG. 2.1. Postero-anterior roentgenogram showing a partially calcified cystic lesion in the left apex and increased radiolucency of the left lung.
B. Bronchogram demonstrating diffuse cylindrical bronchiectasis.
C. Angiogram showing partial agenesis of the left pulmonary artery.

and oxygen utilization. This was strongly suggestive that the oxygen utilization on the left was diminished due to a lowered perfusion resulting from decreased vascular bed on the side.

The diagnosis was agenesis, incomplete, left pulmonary artery, congenital; cyst of the lung, not classified.

Discussion

The diagnosis of agenesis of the pulmonary artery may be suspected on conventional chest films, but some of the diagnostic criteria are inconstant, and some of the features are also found in other conditions. The points most frequently mentioned in roentgenographic diagnosis are: absence of hilar comma shadow (Danelius' sign) (7); increased radiolucency of the affected lung; diminished volume of the affected lung; decreased size of the hemithorax on the affected side; herniation of the contralateral lung; position of the mediastinum shifted toward the affected lung to compensate for the reduced lung volume; and further shift of the mediastinum toward the affected lung on deep inspiration.

Madoff and associates (1) and others have questioned the value of Danelius' sign. The change in position of the mediastinum to compensate for the reduced lung volume tends to make evaluation of the hilar vascular shadows difficult. Actually, Danelius' sign was not meant to be applied to this entity, but was originally described in connection with a right-sided truncus aorticus solitarius (7). Findlay and Maier (3) state that less prominent than normal hilar shadows may be evidence that a major portion of the pulmonary blood supply is arising from an unusual location, but that it is not an indication, per se, of absence of the pulmonary artery. Reduced pulmonary arterial shadows may be only an indication of pulmonic stenosis. It may also be found in massive occlusion of a branch of the pulmonary artery. Ring and Bakke (25) have pointed out that this finding, relatively common at autopsy, may also be present in patients who survive, and that the absence of associated infarction is explained by the collateral circulation between the bronchial artery and pulmonary circulation. Figure 3 is one of a series in an angiocardiographic study of a patient with this condition.

Increased radiolucency, a manifestation of re-

Fig. 3. Angiocardiogram showing the effect of a thrombus of the left pulmonary artery.

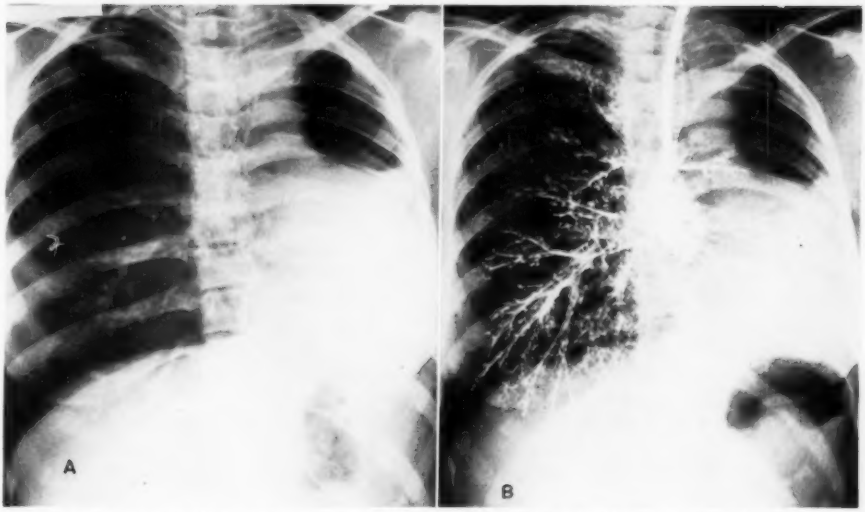


FIG. 4A. Postero-anterior roentgenogram showing agenesis of the left lung. *B*. Bronchogram demonstrating agenesis of the left lung.

duced vascularity, and frequently the most striking feature on routine films, is seen not only in congenital absence of the pulmonary artery, but also in pulmonary stenosis, massive pulmonary arterial thrombosis (figure 3), compensatory emphysema to massive atelectasis, or pulmonary agenesis (figure 4*A*). Several of these conditions present no problem in the differential diagnosis, but when the cause is not apparent it is an indication for further investigation. Madoff and associates (1) and McKim and Wiglesworth (15) have noted that, on histologic section, no structural differences are seen between the two lungs. Both groups point out that, in spite of apparent overdistention of the lung with normal pulmonary arterial supply, no emphysema is demonstrable. The only positive finding so far noted is medial hypertrophy of the walls of the pulmonary arterioles in the affected lung, apparently a manifestation of increased pressure of the collateral circulation (16).

The reduced blood flow in pulmonary arterial agenesis causes a reduction in lung volume and, as a result, a reduction in size of the hemithorax on the affected side. This is a developmental defect and is not found in young infants. This may lead to an erroneous diagnosis, as illustrated in a case reported by Campbell (18). The conventional films on a four-month, cyanotic infant showed the left lung to be smaller and more radiable than the right. The vascular markings were inconspicuous, and the left hilar comma shadow was invisible. Subsequent developments, including angiocardiography, revealed normal pulmonary arteries, but there was a 2-cm. bronchial cyst compressing the left main stem bronchus. This produced a check-valve mechanism, and the lack of pulmonary markings indicating emphysema was misinterpreted as avascularity. McKim and Wiglesworth (15) have noted that, in the newborn and very young infants, the affected lung is of normal size at birth because of the blood supply through the ductus which, when later obliterated, reduces the blood volume and size of the lung and hemithorax on the affected side.

Herniation of the contralateral lung is not a constant finding in all cases. Failure to observe a further shift of the mediastinum toward the affected side on deep inspiration is also inconstant and will be present or absent, depending upon the degree of aeration of the affected lung, fixation of the mediastinum by chronic pulmonary disease, herniation of the contralateral lung, et cetera.

Alexander and his associates (19), Wyman (14), and others state that angiocardiography is necessary to make a definite diagnosis of congeni-

tal absence of the pulmonary artery. It is believed that this is only partially correct and that angiocardiography *and* bronchography, together, are necessary to establish this diagnosis. Attention is called to figure 4. In this case of agenesis of the lung, the angiocardiogram demonstrated a defect in the pulmonary arterial circulation, but without the bronchogram it would be impossible to determine that this defect was due to agenesis of the pulmonary artery, per se. McKim and Wiglesworth (15) have pointed out that simultaneous filling of both lungs in angiocardiography will occur when there is an overriding aorta, and may be confusing even though one branch of the main pulmonary artery is absent. Maier (16) also advises bronchography to establish that a full number of bronchopulmonary segments are present.

There are many reports on the effects of occlusion of a branch of the pulmonary artery. An excellent review of this subject was done by Madoff and associates (1). Virchow first showed that one branch of the main pulmonary artery might be ligated without causing necrosis of the lung if the bronchial blood supply on that side was not also compromised. The physiologic effects of ligation were investigated in animals by Marr, who showed that the occlusion caused a cessation of oxygen uptake and carbon dioxide output. This has been confirmed in man by Carlens and his associates (26). The results of their spirometric studies very closely parallel those so far reported in patients with pulmonary arterial agenesis. However, Maier (16) believes that the network of vessels connecting the two vascular systems in certain congenital anomalies may differ from those produced in surgical or experimental procedures.

It is known that reduction in blood flow through a pulmonary artery, from any cause, results in diminution in ventilation to a varying degree, and that the respiratory function of the affected lung is largely lost. Bloomer and associates (22) produced enlargement of the bronchial arteries in dogs by ligating a main branch of the pulmonary artery, and Bing and co-workers (23) have noted a similar condition in patients with diminished arterial flow in pulmonary stenosis. In patients with congenital heart disease with associated agenesis of the pulmonary artery, the increased bronchial flow, in effect an A-V shunt, is important to survival. This has been pointed out by Maier (16), Campbell (18), and others, and the presence or absence of congenital heart disease must be established before any definitive surgical treatment is considered.

The role of bronchospirometric studies in diagnosis is clear, the abnormal lung showing a marked disparity between ventilation and oxygen consumption. The ventilation of the affected lung tends to be normal or moderately reduced, whereas oxygen consumption is markedly reduced. Although few reported cases have had bronchospirometric study, these findings have been consistent and may be considered diagnostic of the basic arterial defect but not of the etiology (27).

In a report by Flynn and his associates (17) of a case of absence of the right pulmonary artery, the external spirometric studies showed only a reduced vital capacity. Internal spirometric readings showed marked differences in the contribution of each lung to total function. Although 43 per cent of ventilation at rest was performed by the right lung, there was only a 4 per cent oxygen uptake by the right lung with the patient breathing pure oxygen. Arterial puncture was carried out simultaneously with this, and the oxygen saturation of the peripheral blood was found to be in normal range. It was concluded that, since oxygen uptake is related to volume flow of blood, virtually none of the patient's pulmonary arterial stream was directed to the right lung. Had there been normal pulmonary arterial flow without oxygen uptake, defective oxygenation of the peripheral arterial blood would have been expected.

Campbell (16) states that the oxygen uptake and carbon dioxide output are always reduced, sometimes to 1 or 2 per cent of the total, depending on the adequacy of the blood supply through the bronchial arteries and anomalous vessels. Study has clearly demonstrated that this abnormality is due to a pulmonary arterial perfusion disturbance.

Animal experimentation and observation on human subjects have demonstrated several other things which are of practical application. Batts (28) reported a case of an aberrant pulmonary artery arising from the abdominal aorta. In this he noted arteriosclerotic changes not present in the other pulmonary arteries. While he reported this finding as supportive evidence of hypertension as a cause of arteriosclerosis, similar changes have been found rather frequently by other observers. This tendency to degenerative changes in

vessels from the systemic circulation to the pulmonary circulation may be an indication for definitive surgery.

Hemoptysis in patients with agenesis of the pulmonary artery may also be an indication for surgical treatment. This has been explained by Sweet and White (10), Findlay and Maier (3), and others as the result of hypertension in the pulmonary circulation incident to supply from the systemic side of the dual circulation through aberrant arteries or through dilated bronchial arteries. The role of degenerative changes in aberrant vessels in conjunction with, as well as the result of, increased pressure in these vessels must also be considered in the production of hemoptysis. Maier (16) states that the components of the wall of an abnormal systemic vessel are important in determining the amount of blood that is delivered to the lung and, therefore, are related to congestion in the lung. There is not sufficient information of this nature as yet reported to permit evaluation of this factor. Bronchiectasis has been previously noted to be related to agenesis of the pulmonary artery, and this only further compromises the bronchial arteries. Liebow (29) notes that patients with cystic disease or bronchiectasis have enlarged bronchial arteries under systemic pressure, and he believes that this is an explanation for the hemoptysis seen in these patients.

Bloomer and his associates (22) also showed that, when the pulmonary artery is ligated, one-third of the output of the left ventricle may go to the bronchial arteries. This added burden on the heart may result in cardiac failure and may, therefore, be another indication for definitive therapy in patients with pulmonary arterial agenesis.

Another factor bearing upon therapeutic recommendations is the tendency to chronic infection in lungs with defective pulmonary circulation. This is not a manifestation of ischemia, but is the result of structural changes in the bronchopulmonary tree. The bronchi are generally smaller in caliber and more closely spaced. This apparently impedes drainage of secretions and is a basis for recurrent pulmonary infection.

Summary

Two cases of agenesis of a major portion of one of the main branches of the pulmonary artery are presented. Because the embryologic factors are related and because the therapeutic considerations are the same, it is proposed that this type of congenital defect be grouped with those cases in which there is complete agenesis of one of the main branches of the pulmonary artery.

While an increasing number of cases of agenesis in the pulmonary arterial circulation have been appearing in the literature, the information is yet too scanty to draw any very definite conclusions about this anomaly. Nine points are suggested for reporting of cases in the future which, it is believed, are necessary for adequate documentation and evaluation. Diagnosis can only be suspected on conventional films, and confirmation can be had only when angiocardiographic and bronchographic studies are combined.

The pathologic physiology is discussed in some detail, including the correlation of bronchospirometric findings. Indications for definitive therapeutic measures are still uncertain, but must be found by understanding the pathologic and physiologic changes in each case. The indications for surgical intervention, as presently conceived, seem to be: (*1*) left heart failure, (*2*) hemoptysis, and (*3*) chronic infection. An attempt has been made to explain the causal factors in each of these.

Sumario

Agenesia en la Circulación Arteriopulmonar. Presentación de Dos Casos

Preséntanse 2 casos de agenesia de una gran porción de una de las principales ramas de la arteria pulmonar. Por estar relacionados los factores embriológicos y ser idénticas las consideraciones terapéuticas, propónese que se agrupe esta forma de anomalía congénita con los casos en los que existe agenesia total de una de las principales ramas de la arteria pulmonar.

Aunque ha ido apareciendo en la literatura un número cada vez mayor de casos de agenesia en la circulación arteriopulmonar, los datos son todavía demasiado escasos para sacar conclusiones muy precisas sobre dicha anomalía. Sugiérense nueve puntos para la presentación de estos casos en el futuro, que, según parece, son necesarios para adecuada documentación y justipreciación.

Enuméranse los hallazgos radiográficos para diagnóstico, discutiéndose algo a fondo el valor de cada uno, y comprendiendo la correlación de los hallazgos broncospirométricos. En su concepto actual, las indicaciones para la intervención cruenta parecen ser: (*1*) insuficiencia del corazón izquierdo, (*2*) hemoptisis y (*3*) infección crónica.

Se trata de explicar los factores etiológicos en cada una de ellas.

Resume

Agénésie de la circulation artérielle pulmonaire. Rapport sur deux cas

Deux cas d'agénésie d'une portion majeure de l'une des branches principales de l'artère pulmonaire sont présentés. Etant donné que les facteurs embryologiques sont apparentés et que les considérations thérapeutiques sont les mêmes, les auteurs proposent de grouper ce type de défaut congénital avec les cas dans lesquels il y a agénésie totale de l'une des branches principales de l'artère pulmonaire.

En dépit du nombre croissant des cas d'agénésie dans la circulation artérielle pulmonaire qui ont été publiés, l'information est encore trop réduite pour tirer des conclusions très définies à l'égard de cette anomalie. Neuf points sont suggérés pour l'établissement des rapports sur ces cas dans l'avenir, ce qui est considéré nécessaire pour une documentation et une évaluation adéquate.

Les signes diagnostiques radiologiques sont énumérés et la valeur de chacun d'eux est étudiée de façon assez détaillée, y compris la corrélation avec les signes broncho-spirométriques. Les indications pour l'intervention chirurgicale, telles qu'elles sont conçues actuellement, paraissent être: (*1*) la défaillance cardiaque gauche, (*2*) l'hémoptysie, (*3*) l'infection chronique. Une tentative d'explication des facteurs en cause dans chacun de ces cas a été effectuée.

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PULMONARY HISTIOCYTOSIS X WITH DIABETES INSIPIDUS[1]

FERENC PONGOR AND ZOLTAN VIRAGH[2]

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INTRODUCTION

Histiocytosis X or eosinophilic granuloma is a rare disease of unknown etiology, presenting either in localized form or occurring as a disseminated disease. Localized forms have been limited to bone (1), intestine (2), skin (3), and lung (4). The disseminated variances are the Hand-Schüller-Christian and the Letterer-Siwe syndromes. All of these disorders are variations of the same entity according to Farber (5).

CASE REPORTS

Case 1. An 18-year-old white male patient was admitted to the internal medicine department of the University of Debrecen on December 28, 1955. His chief complaints were shortness of breath on slight exertion and easy fatigue. He had always been frail and for this reason had attended school for only three years. At fifteen he noticed that he was constantly thirsty, gradually increasing his fluid intake until he drank ten to twelve liters daily.

The family history revealed that one of his four sisters was receiving antituberculous outpatient treatment and his father was suffering from bronchial asthma. His mother was in good health.

On admission to the internal medicine ward, the roentgenograms of the chest and sella were normal. The daily urine output was 8 to 10 liters, with specific gravity of 1.001, at both the beginning and the end of the test. Vasopressin was administered, and the daily urine output decreased to two or three liters. No side effects were observed, and the patient was discharged with the diagnosis of diabetes insipidus.

On April 19, 1957, the patient was admitted to the tuberculosis ward. He said that he had been unable to do any work since he had left the hospital, he felt weak, and was short of breath. Three weeks before, he had experienced a sharp pain in the left side of his chest and became more short-winded. From the roentgenograms, a diagnosis of spontaneous left pneumothorax was made. In addition to pneumothorax, the roentgenogram revealed a diffuse reticular pattern in both lungs. The trachea and mediastinum were deviated to the left (figure 1).

Dyspnea ceased after the aspiration of 500 ml. of air. The patient's pulse was 78 per minute; blood pressure, 110 systolic, 90 diastolic in mm. of mercury. A Mantoux tuberculin reaction was negative in a dilution of 1:100. Culture of bronchial secretions was negative for acid-fast bacilli. The urine output was 2 to 3 liters daily, with normal laboratory findings. Liver function studies revealed normal results. An electrocardiogram was normal. The hematologic findings were: neutrophils, 51 per cent; lymphocytes, 37 per cent; and monocytes, 12 per cent. Roentgenograms of the skull, spine, and sella were normal. Because tuberculosis could not be excluded, chemotherapy consisting of isoniazid–PAS was started. A roentgenogram after eight weeks showed complete re-expansion of the left lung.

In an attempt to arrive at a definite diagnosis, exploratory thoracotomy was done on November 6, 1957. The visceral pleura appeared thickened and dull, and on its surface there were many gray-black nodules ranging from 1 to 5 mm. in diameter. Biopsy was done of the pleura and the lung. Microscopic examination revealed capillary vascularization of the thickened pleura (figure 2). In the lung parenchyma there were many nodules of various sizes consisting of fibrous connective tissue and capillary vessels. Around these nodules, several lymph follicles with large light hollowed centers and spherical cells could be seen. On the borders of the infiltration, eosinophils were found. In one of the fibrous connective tissue nodules there was a small tissue defect forming a minute cavity filled with erythrocytes. In the wall of this unit there were several nodules consisting of histiocytes, eosinophils, and multinucleated giant cells. Nodules composed of macrophages and a few eosinophils could be detected in the interstitium as well. The alveolar pattern was maintained. In the interalveolar tissue there were erythrocytes, dark pigment, and increased capillary vascularization. The histopathologic diagnosis was histiocytosis.

The patient was discharged and was instructed to return for follow-up examinations.

Case 2. A 30-year-old farmer was admitted to the tuberculosis department of the University of Debrecen on August 22, 1957. There was nothing remarkable in his past history; the family history was also noncontributory. His present illness began two days prior to admission with a sudden

[1] Cooperative study from the University of Debrecen, Department of Tuberculosis, Debrecen, Hungary; and the U. S. Veterans Administration Hospital, Tuberculosis Service, Wood, Wisconsin.

[2] Present address: Veterans Administration Center, Waukesha Division, Waukesha, Wisconsin.

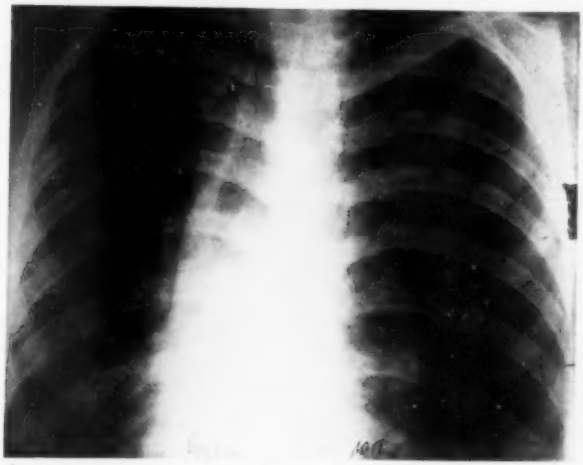

FIG. 1. *Case 1.* Left partial pneumothorax. Mediastinum shifted to the right.

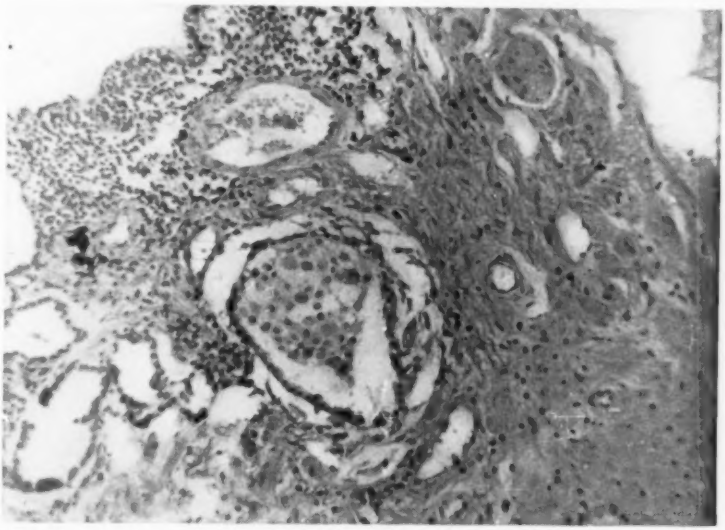

FIG. 2. *Case 1.* Low-power photomicrograph showing the alveoli filled with endothelial cells and histiocytes. (Hematoxylin and eosin stain)

shooting pain in his left chest, a severe coughing spell, and a choking sensation. He was sent to the tuberculosis dispensary, where a diagnosis of spontaneous pneumothorax was made. The roentgenogram showed a 2.5 cm. ribbon of air surrounding the left lung and diffuse miliary, fibrotic-appearing infiltration in both lungs; the mediastinum and trachea were shifted to the right (figure 3). The right lung was moderately emphysematous. After a single aspiration of 1,500 cc. of air, the patient's dyspnea was relieved. No further desufflation was necessary.

Bronchial secretions were negative for acid-fast bacilli on five successive cultures. A Mantoux reaction was positive in 1:10,000 dilution. The erythrocyte sedimentation rate was 10 mm. in an hour. Blood pressure was 110 systolic, 100 diastolic in mm. of mercury. Hematology was not remarkable. The daily output of urine was 10 to 12 liters with specific gravity of 1.002; otherwise it was

within normal limits. Vasopressin was given and the urine output decreased to 2 to 3 liters daily.

The left lung had re-expanded after a period of time, when suddenly a spontaneous pneumothorax developed on the right side.

On November 13, 1957, a right thoracotomy was performed for the purpose of a lung biopsy. On the lung surface, especially on the posterior surface of the upper and lower lobes, many grayish nodules 1 to 2 mm. in diameter were seen. On the base of the upper lobe, in the fissure, there was a thin-walled, broken bulla about 15 mm. in diameter. A small wedge of lung tissue was excised, and the chest was closed after dusting the pleura with iodized talcum to promote adhesions.

In the alveoli there were erythrocytes and

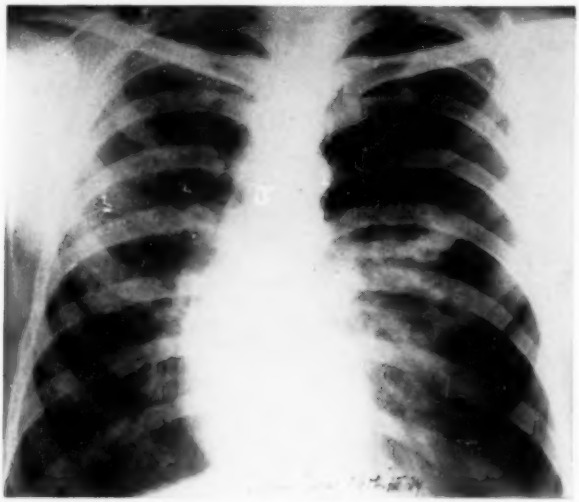

FIG. 3. *Case 2.* Complete left pneumothorax, bilateral diffuse miliary infiltrations.

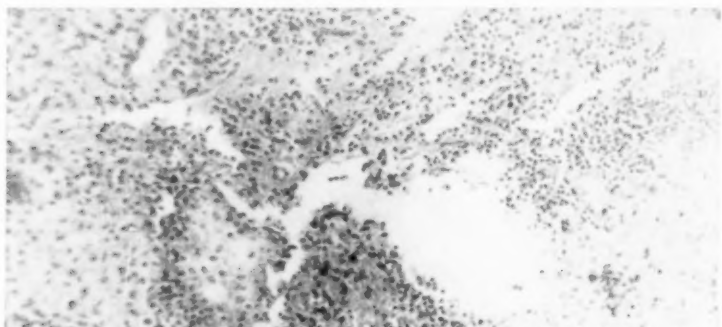

FIG. 4. *Case 2.* Low-power photomicrograph showing histiocytes in the alveoli; eosinophils and lymphocytes in the interstitium. (Hematoxylin and eosin stain)

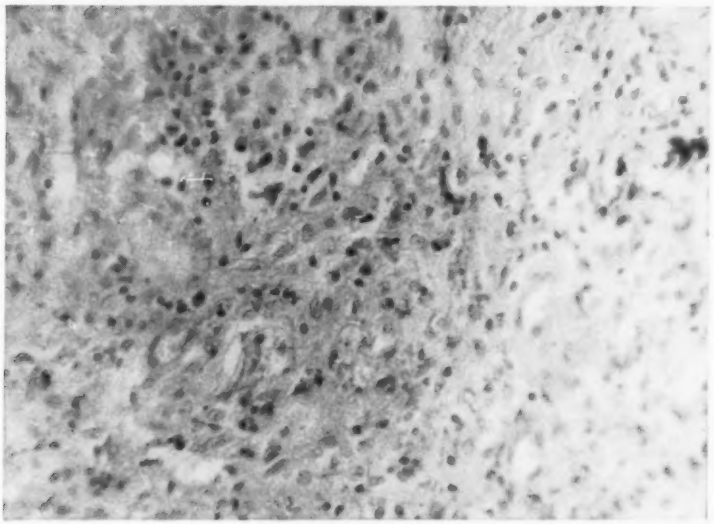

FIG. 5. *Case 2.* High-power photomicrograph of an intra-alveolar granulomatous focus. (Hematoxylin and eosin stain)

detached endothelial cells. The walls of the alveoli were thickened. Several nodules were found in the interstitium consisting of large polygonal cells with light plasma (figures 4 and 5). The nuclei were oval or round, faintly stained; their chromatin was somewhat coarse, the nucleoli were discrete. Also among these cells were eosinophils, neutrophils, and lymphocytes. Many fibroblasts were seen on the periphery of these nodules which penetrated the stroma in places. Nodules were also found in which collagen connective tissue was dominant. Round-cell infiltration was noted in these nodules. On other parts of the specimen around the widened vessels and small bronchioli, follicle-like lymphocytic conglomerations were seen, among which some dark pigment was noted, possibly hemosiderin. The cytoplasm of the large, light cells contained sudanophilic material. The diagnosis was histiocytosis.

After the right lung re-expanded, the patient left the hospital. No treatment was given.

DISCUSSION

Review of the Literature

The simplest form of histiocytosis, "solitary granuloma of bone simulating primary neoplasma," was described by Otoni and Ehrlich (6) in 1940. Simultaneously the term "eosinophilic granuloma" was applied by Lichtenstein and Jaffe (1). The first two cases of eosinophilic granuloma with pulmonary involvement only, documented by lung biopsy, were reported by Farinacci and his associates (4) in 1951. One was the case of a 32-year-old man, admitted with the diagnosis of pulmonary tuberculosis; the other was a young man 24 years of age, who had a differential count of 10 per cent eosinophils. Diffuse pulmonary infiltration was noted roentgenographically in both cases. Thoracotomy revealed numerous small nodules on the pleura, and similar nodules were palpable throughout the lung parenchyma. A biopsy was done, and on microscopic examination large histiocytes and eosinophils were seen. As a characteristic feature, pulmonary fibrosis was found.

May, Garfinkle, and Dugan (7) published a report of three cases. The first patient, a 40-year-old man, underwent lung biopsy, and, when malignancy was suspected on frozen section, lobectomy was performed. The malignancy was not confirmed by histopathology and a diagnosis of histiocytosis was made. Their second patient was a woman, 28 years of age, who had a hazelnut-sized growth on the left ninth rib which was resected. The histopathology was eosinophilic granuloma. Roentgenograms of the lung showed miliary infiltration, and in retrospect it was decided that the same lesion had been present on the first film. The finding in the lung biopsy specimen was identical with that in the resected rib. The third patient, also a woman, 36 years of age, was admitted to the hospital with a diagnosis of spontaneous right pneumothorax. By exploratory thoracotomy, a broken bulla was found on the lung

surface, and numerous small nodules were palpable in the parenchyma. The histopathologic diagnosis was eosinophilic granuloma. All three patients also had diabetes insipidus and mild exophthalmus.

Mazzitello (8) reported two cases of eosinophilic granuloma of the lung without associated skeletal or visceral lesions. Grant and Ginsburg (9) made the diagnosis of eosinophilic granuloma (honeycomb lung) likewise by lung biopsy. Their patient was a 32-year-old male who also suffered from diabetes insipidus. Virshup and Goldman (10) found extensive bilateral infiltration in the lungs of their 15-year-old male patient; their first impression had been far advanced tuberculosis. Here, too, lung biopsy established the correct diagnosis.

Childres and associates (11) reported the case of a 24-year-old male patient who was thought to have pulmonary tuberculosis although the bronchial secretions were negative for acid-fast bacilli. A roentgenogram revealed bilateral pulmonary disease. Exploratory thoracotomy was done, and the histopathologic diagnosis was eosinophilic granuloma. After the diagnosis was established, roentgenographic examination of the skeleton revealed a discrete rarefaction of the right femur. Curettage and biopsy were done, and the bone lesion proved to be eosinophilic granuloma. Roentgen therapy was employed for both the lung and bone lesions with good result.

Five cases—all male patients—were reported by Arnett and Schulz (12) in 1957. None of the five patients had any skeletal or other visceral lesions. The writers concurred with Mazzitello (8) that primary eosinophilic granuloma should be considered as a separate disease entity. At present, Rose (13) is treating a young woman (the third recorded female patient) with roentgen therapy. The diagnosis was made by lung biopsy. No extrapulmonary lesion or symptoms other than those related to the lungs were manifested.

The first case of a non-white patient was reported by Thompson, Buechner, and Fishman (14). Their patient was a 42-year-old Negro male with honeycomb appearance in both lungs. No skeletal or other visceral lesions were found. After establishing the diagnosis of eosinophilic granuloma of the lungs by lung biopsy, treatment with prednisone was administered with good result.

It is believed that with the report of Weiss and Johnston (15) all of the published cases of pulmonary histiocytosis confirmed by lung biopsy have been reviewed. Their patient was a 32-year-old male who had spontaneous pneumothorax first on the left, later also on the right side. Bilateral thoracotomy was performed and the diagnosis was established. Initially the disease was confined to the lungs, but ten months later the patient developed diabetes insipidus.

There is no agreement on the terminology for this disorder. The two terms "eosinophilic granuloma of the lung" and "pulmonary histiocytosis X" are used interchangeably to denote a granulomatous disease characterized by the presence of eosinophils in or around the granulomas. The existing confusion concerning the name of the malady under discussion begins with the histology and histopathology.

It is known that granulomas develop by the fusion of histiocytes (Aschoff) which are also called clasmatocytes (Ranvier), resting wandering cells, polyblasts (Maximow), macrophages (Metchnikoff), and several other names. The histiocytes lining the walls of the hemopoietic organs, i.e., the spleen, liver (Kupfer cells), bone marrow, and lymph nodes, form the reticuloendothelial system (Aschoff-Landau). But the "heart failure cells," the "dust cells," the "scavenger cells," the epithelioid and giant cells of the tubercle—itself a granuloma—are also histiocytes in origin.

Histiocytes are present in the chronic inflammatory processes, and have the ability to phagocytose certain substances. In some metabolic disorders, e.g., in the Niemann-Pick's disease and Gaucher disease, the histiocytes are distended with lipid, and this same feature is noted in the Hand-Schüller-Christian syndrome which is considered to be of the same etiology as eosinophilic granuloma.

In 1953, Lichtenstein (16) coined a new name, histiocytosis X, which would include localized eosinophilic granuloma of bone, the acute or subacute Abt-Letterer-Siwe syndrome with or without destructive bone lesions, and the disseminated chronic or subchronic Hand-Schüller-Christian syndrome. Rubin (17) recognizes these three disorders as inflammatory histiocytosis and mentions also eosinophilic granuloma limited to the lungs. Lightwood (18) applied the term "histiocytic reticuloendotheliosis." Bodian (19) thought that all of these syndromes might be histiocytic neoplastic diseases.

The three disorders mentioned and also the localized pulmonary form can be considered as inter-related manifestations of the same disease process, probably inflammatory in origin, although some writers discuss them among the

metabolic disorders; others group them with the diseases of the reticuloendothelial system.

The clinical symptoms of pulmonary histiocytosis tend to be minimal, roentgenograms are not characteristic, and the laboratory findings are not specific. Bronchoscopy occasionally reveals diffuse inflammation; scalene node biopsy is not pathognostic. The correct diagnosis can only be established by lung biopsy. The differential diagnosis includes almost all of the granulomatous diseases and the pneumoconioses.

Spontaneous pneumothorax develops not infrequently and is a logical mechanical sequel well explained by the histopathology, in which a cyst or small cavity formation within the granuloma is frequently described. If this small cavity is close enough to the surface of the lung, it may rupture and cause the spontaneous pneumothorax.

It seems that histiocytosis affects the male most frequently. Roentgen therapy, steroids, and antimicrobial drugs have been employed in its treatment. In cases of approachable bone lesions, resection is feasible. The results of these therapeutic experiments vary from "good" to "no change." In other cases in which no treatment was given, spontaneous regression has occurred, making evaluation of therapy difficult.

Conclusions cannot be drawn from the limited number of case reports and the meager knowledge of this entity; yet there can be little doubt that the disorder is a systemic disease, which may appear also as a solitary lesion. It seems logical to agree with Mazzitello (8) that, similarly to primary eosinophilic granuloma of bone, the disease localized in the lung should also be recognized as a distinct nosologic entity. It is highly questionable, however, whether it qualifies as a "primary" condition since it is entirely reasonable to assume that the eventually coexisting diabetes insipidus, in at least seven of the twenty cases reviewed, is of the same etiology.

Summary

Two cases of pulmonary histiocytosis with coexisting diabetes insipidus are reported. The diagnoses were established by lung biopsy. Spontaneous pneumothorax occurred in both patients.

Sumario

Histiocitosis Pulmonar con Diabetes Insípida

Preséntanse 2 casos de histiocitosis pulmonar con diabetes insípida coexistente. Se estableció el diagnóstico con la biopsia pulmonar. En ambos enfermos ocurrió neumotórax espontáneo. Preséntase además una reseña de la literatura.

Resume

Histiocytose pulmonaire X avec diabète insipide

Deux cas de d'histiocytose pulmonaire avec coexistence de diabète insipide sont rapportés. Le diagnostic a été établi par la biopsie pulmonaire. Un pneumothorax spontané s'est produit chez les deux malades. L'article comporte également un compte-rendu de la littérature pertinente.

Acknowledgments

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(11) Childres, J. H., Middleton, J. W., and Schneider, M.: Eosinophilic granuloma of femur and lungs: A case report, Ann. Int. Med., 1955, *42*, 1297.

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Editorial

The Tuberculous Alcoholic Before and During Hospitalization[1]

The inhabitants of "Skid Row" constitute an increasingly recognized but poorly understood minority group which is of mounting concern to those agencies whose function it is to detect, control, and treat tuberculosis. The members of this subgroup of American society are predominantly homeless males of at least middle age. They tend to work only sporadically. They move from one Skid Row residence to another within a city, or from the Skid Row of one city to that of another, with occasional forays into rural areas to work. These men are recognizable, not only by their addresses, but also by their shared customs, patterns of behavior, ethical systems, and language. Their relationships with other members of this group and with outsiders have a similar structure. They also share an orientation toward life, other people, and the institutions of American culture. These shared elements differ markedly from the predominant culture of America.[2-5]

The alcoholics are a subgroup within the Skid Row minority group.[6] Their participation in the Skid Row culture and their relationships with others on and off Skid Row pivot around drinking, the problems associated with drinking, and the adjustments which they, like all severely chronically ill people, make to their illness. For the Skid Row alcoholic, alcoholism is not only an illness from which he suffers and to which he makes adaptations, but it is also the basis for his total way of life.

The problem of differentiating alcoholics from nonalcoholics is the same, regardless of where they are located in social space. E. M. Jellinek and others have described the symptoms which alcoholics experience in association with drinking behavior and the usual course of this chronic illness.[9-13] On the basis of these symptoms a diagnosis can be made.[14] These symptoms range from drinking on arising, recurrent blackouts, and increased tolerance for alcohol at one end of the continuum to "the shakes," hiding bottles, drinking non-beverage alcohol, and suicidal thoughts at the other extreme.

Agencies concerned with the detection and control of tuberculosis are becoming increasingly aware that this minority group of Skid Row alcoholics contains a large proportion of the still undetected and untreated cases of active tuberculosis. In recent years several studies have pointed to this conclusion. Routine roentgenographic examinations of residents of a Salvation Army Social Service Center's clients in Minneapolis, 70 per cent of whom were alcoholics, showed a rate of tuberculosis fifty-five times the rate for the general population of the city in the same year.[15] The investigators considered that the rate of 22.2

[1] This study has been supported in part by the U. S. Public Health Service and the Firland Research Fund.

[2] In our studies at Firland Sanatorium in Seattle, we have used the address at the time of hospital admission as our index of membership in the "Skid Row" culture. The incidence is extremely low of men having a Skid Row address and yet not belonging to the Skid Row culture. The transient who is not a "Skid Rower" tends to reside in an area off Skid Row. For purposes of our study, Skid Row was defined as five census tracts which had equivalent types of housing, population, mobility and occupational patterns, and which were agreed upon as constituting Skid Row by those who called themselves Skid Rowers and agency personnel concerned with providing services for the Skid Row area.

[3] Rubington, E.: Ann. Am. Acad. Pol. & Soc. Sc., 1958, *315*, 65.

[4] Jackson, J. K., and Connor, R. G.: Quart. J. Stud. Alcoh., 1953, *14*, 468.

[5] Pittman, D. J., and Gordon, C. W.: Monograph of Yale Center of Alcohol Studies, No. 2, The Free Press, Glencoe, Illinois, 1958.

[6] In a study of 100 consecutive admissions to Firland Sanatorium from Skid Row, more than four-fifths were alcoholics.[7]

[7] Jackson, J. L.: No. 30. The Problem of the Alcoholic Tuberculous Patient, in Personality, Stress and Tuberculosis, International Universities Press, New York, 1956.

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[13] Bacon, S. D.: Social Settings Conducive to Alcoholism in *Manual on Alcoholism*, American Medical Association, Chicago, 1957, pp. 60-74.

[14] In our research, alcoholism has been defined in terms of this symptom pattern (*see*[7]).

[15] Jones, H. W., *et al.*: J.A.M.A., 1954, *155*, 1222.

new cases per 1,000 was an underestimate of the incidence of active tuberculosis in the Skid Row group. They state: "In a survey that covered not only the younger groups such as those applying to rehabilitation centers but the entire Skid Row population, including the older, more permanent residents, the rate would be higher." Ottenberg[16] reported on a survey of men jailed for drunkenness in Philadelphia during a six-week period. The rate of proved active cases was 18 per 1,000. Ottenberg also believes that this is an underestimate, as no follow-up study was possible on 30 per cent of those whose films were read "probably active" and 58 per cent of those read "activity indeterminate." Ould[17] reported that chest roentgenograms of Fresno jail inmates yielded ten times the expected rate of active tuberculosis. In Seattle, the tuberculosis rate for those jailed for drunkenness is midway between that of Philadelphia and California.[18]

Finding the Skid Row residents with active tuberculosis is not the only problem. Those agencies concerned with the treatment of this illness are aware that there are disproportionate difficulties involved in persuading these men to accept hospitalization and to persist in treatment.

Skid Row alcoholics have no more extensive tuberculosis than other patients. Studies of alcoholic patients discharged from Firland Sanatorium in Seattle in 1957 and tuberculous alcoholic patients in California[19] indicated that there were no significant differences between alcoholics and nonalcoholics in extent of disease. Both the Firland and California studies further indicated that tuberculous alcoholic patients did not differ from tuberculous nonalcoholic patients in the number of nontuberculous diagnoses upon discharge.

Despite the similarities between the two groups in extent of illness, alcoholics had more complications during treatment[19] and experienced more relapses after discharge. At Firland, 23.2 per cent of male alcoholic patients are readmitted with active tuberculosis, compared with an over-all readmission rate of 7.3 per cent. In the California study,[19] 66 per cent of the alcoholics had more than one hospitalization for tuberculosis, compared with 40 per cent of the control group. Because of their transiency and relative inaccessibility to detection techniques, the numbers of male Skid Row alcoholics readmitted to Firland is probably far from a complete enumeration of the number who suffer a reactivation of tuberculosis.

In addition to having a more complicated treatment course and prognosis, Skid Row alcoholics constitute the largest proportion of patients who have difficulties in relating to staff and to other patients and who react to hospital routines with marked discomfort or flight. In 1957, 88 per cent of nonalcoholic male patients were discharged from Firland with medical approval, contrasted with 59 per cent of the alcoholic males. In the California study, 59 per cent of the alcoholics had one or more unsanctioned discharges, compared with 23 per cent of the controls.[19] The Skid Row alcoholics' lack of conformity in the hospital milieu makes effective treatment of their tuberculosis very difficult. These men follow treatment regimens incompletely or sporadically, even when they remain in the hospital.

After discharge, they come to the outpatient clinics infrequently at best. Most return to their pre-hospital Skid Row alcoholic way of life, but at a lower level of social adjustment than before hospitalization for tuberculosis. An alarming number die of other illnesses, in accidents, and by suicide. Many are admitted to mental hospitals within a short time after their discharge. Many become almost permanent residents of the local jails. The number of these men who attempt to work again is infinitesimal. The number of Skid Row alcoholic patients discharged from a sanatorium in a year who recover from alcoholism could be counted on one hand.

It is proper to explore some of the factors which contribute to this over-all picture. As alcoholic patients are discharged from our hospitals in better physical health than they have enjoyed for years, and as they have spent up to a year almost entirely removed from drinking in an atmosphere oriented towards rehabilitation and among "respectable" people, better results would be expected. Two control groups, one of nonalcoholic tuberculous Skid Row patients and one of nontuberculous but institutionalized Skid Row alcoholics,[20] showed considerably better treatment outcomes. A good many more of each control group now lead normal healthy lives.

When recent life experiences of alcoholics who became tuberculous were compared with those of alcoholics who are nontuberculous, the poor results were even more surprising. A significantly greater proportion of those who became tuberculous had been sufficiently troubled by their alco-

[16] Ottenberg, D. J.: TB on Skid Row, Bull. National Tuberculosis Association, June, 1956.

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[18] Fountain, J.: Personal communication.

[19] Hummel, J.: The Alcoholic Tuberculous Patient, California Tuberculosis and Health Association, 1956 (mimeographed).

[20] Jackson, J. K., *et al.*: The Seattle Police Department Rehabilitation Project for Chronic Alcoholics: Description and Evaluation, Federal Probation, 1958, *22*, 36.

holism that they had tried to change it.[7] In their attempt, they had realistically cut themselves off from the Skid Row way of life, knowing that maintaining sobriety and remaining a Skid Rower are almost mutually exclusive.[3,21] The attempt had failed. Drinking had resumed.[22] As one of the prerequisites to recovery from alcoholism is the recognition of alcoholism as an inadequate adaptive technique, it would have been thought that a large proportion of tuberculous alcoholics entered the sanatorium in a state of mind conducive to recovery from alcoholism.

Still other factors would contribute to this expectation. Tuberculosis and prolonged hospitalization are seen by the alcoholic patient as a major threat to his personality integration. One of the strongest underpinnings of his feelings of worth has been his illusion of independence and self-sufficiency. This illusion has been based largely on his physical health and on his ability to take care of himself without help from others. He feels that tuberculosis threatens to make him dependent on others. One of his major adaptive techniques in the face of severe stress has been mobility. He feels that tuberculosis may put a stop to his ability to move.

In addition, for the first few weeks in the sanatorium he is able to understand how his alcoholism contributed to his present condition. His usual defenses against full awareness of the extent of his alcoholic problem are extremely shaky at this time.

During his first weeks in the sanatorium, he is particularly vulnerable. He is totally removed from his usual social context and placed in a totally foreign environment. As mentioned earlier, alcoholism is not only an illness to the Skid Rower. It is the basis for membership in a well-integrated sub-society which has its own way of life, its own values, its own view of the world and of the individual's place in it.[4] When a member of this minority group enters the sanatorium, he no longer has the emotional support of his Skid Row associates. He can no longer count on his own society's view of the world to account for what has happened to him. He cannot use his usual structuring of life to assist him in rebuilding his personality defenses. He is unable to use drinking, sociability, or mobility to avoid his problems. He cannot regulate the extent or quality of his relationships with others as he did in Skid Row. He cannot move toward or away because he is confined to bed. He cannot use his one other technique of regulating relationships with nonalcoholics, i.e., fighting them. Temporarily he depends on these nonalcoholics for his continued existence.

At this stage, when he would appear to be most amenable to help, his alcoholism is overlooked. He is treated as if tuberculosis were his only illness and as if his alcoholism were a thing of the past or merely a prognostic index. To the patient, alcoholism is the major problem—alcoholism as an extensive and organized syndrome in which excessive drinking is only the most visible symptom. Admittedly, his tuberculosis is also of some concern to him. However, the major part of his concern is not about tuberculosis per se, but about its meaning in relation to the total syndrome of alcoholism—its meaning in relation to his personality, his way of life, his way of thinking and behaving, his way of relating to other people, and his place in the world.

His Skid Row way of life has been so different from the hospital situation that he finds it difficult to understand the sanatorium way of life and to understand how he is expected to behave. In this situation he relies on the staff to interpret the hospital to him and to guide his behavior. As a result, he tends to be a "good patient" who cooperates with treatment.

As the patient gets used to the sanatorium setting, begins to feel well, and re-establishes his relationships with other Skid Rowers, *his* problems diminish. The *staff's* problems with him begin.

As he becomes reintegrated into his Skid Row group, it replaces the staff as the reference point for his attitudes and behavior and for his perception of the hospital situation.[3,23] He begins to deny all problems of which he was previously aware, including his alcoholism. Any nonalcoholics who mention alcoholism to him are now thought to be making unjust accusations. Any staff demand which his group interprets as an infringement on his right to be self-determining is rejected. Except when he is acutely ill, dependency on the staff is regarded by his group as close to traitorous behavior, as is continued cooperation with it. In rebuilding his defenses, he again values self-sufficiency and independence, and expresses this by assertiveness which is approved by his group. His behavior would indicate that it becomes more

[21] RUBINGTON, E.: Panhandling and the Skid Row subculture, paper delivered at the 53rd annual meeting of the American Sociological Society, Seattle, Washington, August 28, 1958.

[22] It is also interesting to note that the subjective awareness of the symptoms of tuberculosis appeared in this setting.

[23] JACKSON, J. K.: No. 31. Some Aspects of Sanatorium Adjustment Difficulties of White, Male Skid Road Alcoholic Tuberculous Patients, in Personality, Stress, and Tuberculosis, International Universities Press, New York, 1956.

important to be assertive than to recover from tuberculosis. He mills around with other Skid Rowers in an apparently aimless manner, re-establishing his use of mobility and sociability as adaptive techniques.

As his behavior raises problems for the staff, conflicts arise. The staff tries to resolve them by straightforward discussion of the problem, manipulation, exhortation, or by punitive measures. This is a familiar situation for the member of Skid Row and he has in his cultural background all of the patterns of behavior to cope with it.[3, 22] He denies his own role in any problem. All his old definitions of the nature of nonalcoholics are dusted off and brought forth. He sees problems as being due to unreasonableness, hypocrisy, inconsistency, et cetera, of nonalcoholics. His group solidly agrees and gives him massive emotional support. Effective communication between staff and patient breaks down almost completely. From this point onward the patient and his group watch the staff for proof that their assessment of nonalcoholics is accurate. Problems multiply or an uneasy truce is maintained.

With the re-establishment of so many patterns of Skid Row behavior and relationships and with the setting up of a hostile out-group of staff against whom one must assert oneself, drinking is likely to begin. Drinking again becomes a right to be defended, as well as an expression of identification with his group and of "separateness" from all others.

Now the word alcoholism is heard among the staff. Efforts may be instituted to help the patient with his "drinking problem." By now, however, the patient is almost completely insulated against such efforts. In most respects his environment is the same environment which has supported his alcoholism in the past. He has re-established his most comfortable way of life and is well on the way to returning to it after hospitalization.

Unfortunately, other aspects of institutionalized life have impaired his ability to survive without help when he does return to this way of life. The most obvious is his dependence on regular room and board. His poorer health makes it more difficult to be transient. He is no longer satisfied with dirty and inadequate facilities. The staff of the sanatorium, just by virtue of having been a visible and constantly present out-group, has played a role in maintaining his Skid Row personality integration. He finds it difficult to maintain his personality integration without this foil. After a year of sobriety, his drinking is even more severe after it is resumed.

* * *

The research has not indicated what to do to change this situation. However, it would suggest that help be given with alcoholism for, without this help, the patient cannot recover. It has been suggested that this help be instituted on admission to the hospital, rather than after many months. To do this effectively, staff members will have to equip themselves with considerably greater knowledge about alcoholism and about the agencies experienced in treating alcoholism.[21]

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[21] For example, Alcoholics Anonymous, The National Council on Alcoholism, Community Alcoholism Clinics or Information Centers, American Medical Association Committee on Alcoholism, Counselling Agencies, et cetera.

Notes

DISTINGUISHING MYCOBACTERIA BY THE NIACIN TEST

A Modified Procedure

Distinguishing *Mycobacterium tuberculosis* from other slow-growing nonpigmented organisms is a difficult but important obligation of the bacteriology laboratory. Production of niacin (nicotinic acid) is a newly recognized distinctive feature of human tubercle bacilli.[1-2] Its determination gives promise of providing assistance in this differentiation. Bovine tubercle bacilli produce little, if any, niacin; and most other mycobacteria, such as *M. fortuitum*, *M. avium*, and *M. phlei*, are completely niacin-negative by the usual testing procedures. Unclassified strains of mycobacteria of clinical origin also have been reported to be negative.[3, 4]

In 1956, Konno outlined a procedure applicable to the clinical laboratory for the crude testing of niacin production by mycobacteria.[5] This method (hereafter referred to as the Konno direct test) involves the transfer of bacilli to a test tube containing 4 per cent alcoholic aniline. Addition of 10 per cent cyanogen bromide results in the formation of a yellow color if niacin is present in adequate concentration. Although the principle of this method is well established, false-negative or doubtful results are often obtained. Commonly the yellow color is faint and is seen only in an area close to the bacilli, which may themselves have some color, thus making interpretation uncertain. All cultures which give negative or doubtful reactions must be re-tested by subculture to a liquid medium, a procedure which is time-consuming. This report describes a modification of the Konno test which appears to eliminate false-negative results and provides certain other advantages.

This test is applicable to mycobacterial cultures on solid medium. The colonies should be mature. Uninoculated medium should be tested as a control.

Sterile water or physiologic saline (0.5–1 ml.) is added to the culture tube or bottle. If fluid is already present in the culture tube, this fluid may be used directly for the test.

The culture is placed so that the fluid is layered over the mycobacterial growth. Niacin, if present, will be extracted into the fluid within a few minutes. Five minutes is ample extraction time for the usual culture; if it is anticipated that there may be only a small amount of niacin present, the extraction time should be prolonged to thirty minutes.

An 0.25–0.5 ml. portion of the fluid is removed for the test. If the bacteria to be tested are colored and become dispersed in the test fluid, they should be removed by settling, centrifugation, or dropping the suspension onto a pledget of cotton in the upper part of the test tube. Equal quantities of the test fluid, 4 per cent alcoholic aniline and 10 per cent aqueous cyanogen bromide, are used. (The fume hood must be used for the latter step.) If niacin is present, a yellow color develops promptly throughout the entire solution.

An alkaline germicide (cresol with sodium hydroxide) is added to check tear gas formation before discarding tests.

Alternate method to avoid tear gas production: Tear gas exposure and the need for a fume hood is eliminated by substituting 1 per cent aqueous potassium or sodium cyanide for the cyanogen bromide, and 5 per cent aqueous chloramine T for the aniline in the procedure above.[6] Limited experience indicates that the color obtained with this method is less intense than that with cyanogen bromide.

A distinct yellow color produced by mycobacteria from human subjects indicates human tubercle bacilli. A faint color is obtained with human tubercle bacilli only if the colonies are sparse or poorly developed, as may occur in young cultures or on medium not supporting good growth. A few rarely encountered species of mycobacteria may give weak niacin reactions (*see* below).

A negative result (absence of yellow color) strongly suggests that the organisms are not human tubercle bacilli. Bovine strains will be either negative or weakly positive. Ordinarily in areas such as the United States, where bovine strains are rare, a negative test resulting from a slow-growing nonpigmented culture indicates a *Group III* non-photochromogen.

Regardless of the results of niacin tests, corroborative evidence should be sought from other

[1] Pope, H., and Smith, D. J.: Am. Rev. Tuberc., 1946, *54*, 559.

[2] Konno, K.: Proc. Imp. Acad. Japan, 1953, *29*, 289.

[3] Bönicke, R.: Jahrb. Borstel, 1956–7, *4*, 43, 1957.

[4] Konno, K., *et al:* Am. Rev. Tuberc., 1958, 77, 675.

[5] Konno, K.: Science, 1956, *124*, 985.

[6] Bönicke, R., and Lisboa, B. P.: Tuberkulosearzt, 1958, *6*, 380.

TABLE 1
TESTS FOR NICOTINIC ACID IN CULTURES OF MYCOBACTERIUM TUBERCULOSIS

	Number of Tests		
	Positive	Doubtful	Negative
Direct Konno test....	90	14	69
Modified procedure...	166	2	2

Most strains tested by the modified procedure were not the same as those tested by the Konno method.

biologic characteristics, such as colony form, dispersibility, cord formation, catalase and peroxidase activity, and drug susceptibility. All of these have value, but, in the writers' opinion, none is easier, more clear-cut, or more specific than this modified Konno test.

A comparison of results of the modified procedure with the direct Konno test: The initial results with the two methods are given in table 1. The cultures of *M. tuberculosis* were all authentic tubercle bacilli, either patient-strains or standard laboratory strains. Removal of bacteria or aqueous extract in the performance of one method made a culture unsuitable test material for the alternate procedure. With the modified procedure, all tests of niacin production by well-established cultures of *M. tuberculosis* were distinctly positive. Two negative and two doubtful results were obtained with cultures which had minimal growth and dried-up medium. With the direct Konno procedure, on the other hand, 48 per cent of the 173 tests were negative or doubtful. The causes for these false-negative tests are not fully known. However, it is probable that often insufficient niacin is contained in the transferred bacilli to give a detectable yellow color.[7-8] By the modified procedure it appears probable that niacin is extracted not only from the bacilli, but also from the *medium* under the colonies. The uniformly strong positive results with the present method, in contrast to the weak or often negative results with the other method, suggest that much niacin must be taken up and held by the culture medium.

Cultures on the following media were tested by the modified method:

American Trudeau Society medium from four different laboratories
Löwenstein-Jensen medium from three different laboratories
Steenken and Smith medium from two different laboratories
7H9 agar base medium
Tarshis blood-agar medium

A definite yellow color was produced by *M. tuberculosis* growing on each medium except the blood medium. Aqueous extracts of this medium were pink, so that the yellow color due to niacin was obscured. Included with the cultures which gave positive results with the modified procedure were old cultures of *M. tuberculosis* left at room temperature and others stored for many months or years at deep-freeze temperatures.

Forty-one strains were tested by both methods and the results were compared. The direct Konno test was performed first on each culture and, since this removed a significant proportion of the bac-

TABLE 2
COMPARISON OF 41 CULTURES OF HUMAN TUBERCLE BACILLI BY TWO METHODS

Result with Initial Konno Procedure	Result with Modified Method	Number of Strains
−	+	11
±	+ or ++	7
+	++ or +++	18
++	+++ or ++++	5

TABLE 3
TESTS FOR NICOTINIC ACID IN CULTURES OF OTHER MYCOBACTERIA

	Number of Tests		
	Positive	Doubtful	Negative
M. bovis (3 strains).....		2	1
M. avium (6 strains).....			6
M. microti.....	1		
M. ulcerans.....	1		
M. marinum.....		1	1
M. batnei.....			1
M. ranae.....			1
M. phlei (2 strains).....			2
M. smegmatis (3 strains).....			3
M. pellegrino.....			1
M. sp. strain Rabinowitsch....			1
Anonymous mycobacteria:			
Photochromogens, Group I..			15
Scotochromogens, Group II..			19
Non-photochromogens, Group III.....			15
Rapid growers, Group IV...			5

Most strains were tested by the modified procedure or by both methods.

[7] GILANI, S., AND SELKON, J. B.: Tubercle, 1958, *39*, 396.

[8] MAREK, A.: Beitr. Klin. Tuberk., 1957, *117*, 529.

terial growth, advantage was given this test. Nevertheless, as shown in table 2, the modified method consistently gave more distinct results, i.e., a deeper yellow color. All 41 strains were positive by the modified procedure, but 18 were doubtful or negative by the direct Konno test.

Of the other mycobacterial species tested (table 3), only the vole bacillus gave as strongly positive a test as *M. tuberculosis*. This agrees with previously recorded observations.[2, 3, 7] With the modified method a faint yellow color was produced by *M. ulcerans*. An amount of yellow too small to be considered a positive result and designated doubtful (table 3) was produced by *M. marinum* and two of three strains of bovine bacilli. It is important to recognize the existence of these other niacin producers. However, they should seldom be confused with *M. tuberculosis* in the clinical laboratory, since they are easily distinguished by other properties and are rarely found in clinical specimens.

The modified method described herein has a number of advantages. False-negative results appear to be eliminated and the color obtained is much more distinct and easily read. Subculture to a liquid medium is unnecessary. No difficulty is experienced from malachite green in the culture medium. With the direct Konno test, on the other hand, a bit of green medium inadvertently transferred with the bacteria makes interpretation of the test difficult or impossible. The transfer by pipette of an aqueous extract is easier and much more rapid than is transfer of friable bacterial masses. The culture is preserved intact, whereas, by the direct Konno method, one or two tests may use up all of the bacteria in a culture. Finally, the yellow pigmentation of some strains makes interpretation difficult with the Konno test, but does not interfere with interpretation with the modified technique.

In summary: A modified Konno test for niacin is described. This procedure is easier to perform than the direct Konno test, gives more dependable results with cultures on solid medium, and is a useful means for distinguishing human tubercle bacilli from most other mycobacteria.

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THE EFFECT OF TOPICAL HYDROCORTISONE ACETATE OINTMENT AT THE SITE OF INTRACUTANEOUS TUBERCULIN REACTION

Severe reactions to tuberculin injected intracutaneously, as well as the painfulness and itching sometimes accompanying it, are hypersensitivity responses. In recent years the influence of various drugs on the reactions of hypersensitivity has been extensively studied. Whereas opinions on different drugs vary, there is a consensus that the corticosteroids exert an inhibitory action, either total or partial, on reactions of hypersensitivity.

The influence of parenteral or oral cortisone and adrenocorticotropic hormone on the tuberculin reaction has been investigated in guinea pigs[1-4] and in humans.[5] A review of the literature has indicated that the corticosteroids have some usefulness in preventing severe reactions to tuberculin and the concomitant necrosis. However, the manner of administration of the drugs as reported in these studies is not suitable for practical prevention of undesirable effects of tuberculin hypersensitivity. Oral or parenteral administration appears to diminish not only the undesirable side effects, but the diagnostic value of the reaction in general.

Goldman and co-workers[6] maintained that a tuberculin type of inflammation could be inhibited to a certain extent by local injection of hydrocortisone. However, injection of hydrocortisone at the site of the test can cause too marked a change in the diagnostic features of the reaction to be of practical value.

It is well known that the itching and pain and the unsightly nature of a markedly positive reaction to an intracutaneous tuberculin test cause considerable distress. These undesirable side effects of the test may result in reluctance on the part of the general public to accept repeated testing, and thus handicap mass surveys using this procedure.

There appears to be general agreement on the absence of diagnostic significance of the necrotic characteristics of a 4-plus reaction as well as the itching and pain which sometimes accompany an intracutaneous tuberculin test. Nevertheless, a review of the available literature has failed to reveal any attempt to prevent or alleviate these unnecessary side effects.

The object of this paper is to report preliminary observations on a hydrocortisone acetate ointment applied locally at the site of tuberculin injection.

The tuberculin used in this study was a phenol-soluble fraction of Old Tuberculin (OT) previously adjusted to be equivalent to OT (Lilly) 1:1,000 solution (10 tuberculin units per 0.1 ml.) by control testing on 20 adult tuberculous patients.

The hydrocortisone ointment used consisted of 1 per cent hydrocortisone acetate, U.S.P., with 0.025 per cent benzalkonium chloride in a petrolatum base.

For comparative studies, in several patients rose water ointment (U.S.P.) was applied at the control site of the OT injection.

The patients used in this study at the National Jewish Hospital at Denver had proved pulmonary tuberculosis. Two patients with known negative reactions to tuberculin were chosen from the cardiac ward of the hospital.

A group of 26 patients were injected with 0.1 ml. of 1:2,000 dilution of the phenol-soluble fraction of OT on each forearm. Immediately after the injection a circular surface of skin (1 inch in diameter) at one of the injection sites was covered with a thin layer of hydrocortisone acetate ointment. Thereafter, both sites were massaged gently. After two minutes of massaging, the patients were permitted to remove what was left of the ointment, but were requested not to wash the injection site for at least one hour or more.

An additional group of 12 patients were injected as described above except that hydrocortisone acetate ointment was not applied at one of the sites until twenty-four hours after the injection, and massage was carried out as above.

In 3 patients the hydrocortisone acetate ointment was not applied until forty-eight hours after the injection as described above.

Finally, in 3 patients, hydrocortisone acetate ointment was applied on one arm twenty-four hours after the injection of OT, whereas, on the other, rose water ointment was used. This procedure was used as a comparative study of the effect of a nonspecific base on itching and painfulness. The rose water ointment was shown, by testing on 3 patients, to cause no change in the size or form of the tuberculin reaction.

The tuberculin reactions were classified according to National Tuberculosis Association standards (1955); and were described as: slight (+), 0.5

[1] Harris, S., and Harris, T. N.: Proc. Soc. Exper. Biol. & Med., 1950, *74*, 186.

[2] Derbes, V. J., *et al.*: Proc. Soc. Exper. Biol. & Med., 1950, *75*, 423.

[3] Sheldon, W. H., *et al.*: Proc. Soc. Exper. Biol. & Med., 1950, *75*, 616.

[4] Osgood, C. K., and Favour, C. B.: J. Exper. Med., 1951, *94*, 415.

[5] Long, J. B., and Favour, C. B.: Bull. Johns Hopkins Hosp., 1950, *87*, 186.

[6] Goldman, L., *et al.*: J.A.M.A., 1952, *150*, 30.

to 1.0 cm. of induration; moderate (++), 11 to 20 mm. of induration; marked (+++), more than 20 mm. of induration; and severe (++++), when a slough and a necrotic reaction were present.

Itching and pain were recorded as present or absent.

The following criteria were adopted for interpretation of the results of this experiment: (*1*) total inhibition of reaction when there was complete absence of erythema and induration as compared with the control side; (*2*) partial inhibition when the induration was less than that of the control reaction; (*3*) absence of inhibition when there was no difference; and (*4*) aggravated when the control site showed a less pronounced reaction than the one treated with hydrocortisone acetate ointment.

The reactions of the group of patients on whom hydrocortisone acetate ointment was applied at the time of the injection are presented in table 1. It is clearly evident that the application of the ointment at this time produced a mild aggravation of the reaction in the majority of patients. Thus, 7 of the 8 patients with control reactions measured as slight had moderate ones on the side with the hydrocortisone ointment. Two of the 12 patients with moderate control reactions had marked reactions at the hydrocortisone ointment site. The ointment, applied at the time of performing the test, did not influence the marked reactions, nor did it prevent a severe reaction with necrosis in one patient. The negative tuberculin reaction in 2 patients was not influenced by the ointment.

Eleven among the 26 patients on whom hydrocortisone acetate ointment was applied at the time of the OT injection complained of painfulness and itching at the site of the ointment-treated test, as compared with no complaints at the control site. Two patients had painful indurations on the control side, with no pain on the one with ointment, and 3 had itching on both arms.

When the ointment was applied to the site of the tuberculin injection after twenty-four hours (table 2), no significant change was observed in the size of the induration among the 6 patients whose control injections caused moderate reactions. On the other hand, a definite reduction was noted in those with marked or severe reactions. Three with marked reactions had moderate reactions at the site of the application of the ointment. Among the 3 with severe reactions, one had a moderate reaction, and in 2 the reactions were attenuated to a somewhat lesser extent. However, a slough was observed eventually in one of them.

Six among the 12 patients on whose test site hydrocortisone acetate ointment was applied twenty-four hours after injecting the tuberculin complained of itching on the control side only. Two had pain at the control site only. One complained of itching at the site with the hydrocortisone ointment, which continued for twelve hours after application of the ointment and then receded. The itching on the control site persisted. The severest reactor had pain in both arms.

The application of the hydrocortisone acetate ointment forty-eight hours after the tuberculin injection did not alter significantly the size of the induration observed seventy-two and ninety-six hours after the test in the patients in this group who had moderate reactions on the control site. The induration subsided equally on both forearms. In the third patient the reaction progressed to a slough on the control side on the third day after the injection, but remained stationary at the site which was treated with ointment.

TABLE 1

INFLUENCE OF TOPICALLY APPLIED HYDROCORTISONE ACETATE OINTMENT AT SITE OF OLD TUBERCULIN INJECTION ON THE SIZE OF THE 48-HOUR INDURATION

Hydrocortisone Acetate Ointment Applied at Time of Test

Type of Control Reaction	Number of Cases	Type of Reaction at Site Treated With Hydrocortisone Acetate Ointment				
		Negative	Slight	Moderate	Marked	Severe
Negative	2	2	—	—	—	—
Slight	8	1	—	7	—	—
Moderate	12	—	—	10	2	—
Marked	3	—	—	—	3	—
Severe	1	—	—	—	—	1

TABLE 2

INFLUENCE OF TOPICALLY APPLIED HYDROCORTISONE ACETATE OINTMENT AT SITE OF OLD TUBERCULIN INJECTION ON THE SIZE OF THE 48-HOUR INDURATION

Hydrocortisone Acetate Ointment Applied 24 Hours After Test

Type of Control Reaction	Number of Cases	Type of Reaction at Site Treated With Hydrocortisone Acetate Ointment			
		Slight	Moderate	Marked	Severe
Slight	0	—	—	—	—
Moderate	6	—	6	—	—
Marked	3	—	3	—	—
Severe	3	—	1	1	1

Hydrocortisone acetate ointment applied forty-eight hours after the test relieved painfulness at the site on which it was applied in 2 of 3 patients. The third patient had no subjective complaints.

Two of the 3 patients who had rose water ointment applied at the control site complained of itching on this side only

* * *

Mildly aggravated reactions were observed at the treated sites among the patients on whom hydrocortisone acetate ointment was applied at the time of the injection. On the other hand, it is evident from the data presented here that the application of the ointment twenty-four hours after a tuberculin intradermal injection at the site of the test has prevented the appearance of severe reactions in most of this group of patients. Moreover, the ointment prevented or alleviated the complaints of painfulness and itching so frequently associated with strong reactions.

Thus, these preliminary studies suggest a method of preventing unnecessarily severe reactions and minimizing undesirable side effects of tuberculin hypersensitivity. The statistically sound proof that this is a desirable and practicable adjunct to tuberculin mass surveys requires application to a large number of subjects by a randomized controlled study.

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CATALASE AND PEROXIDASE ACTIVITIES OF ISONIAZID-SUSCEPTIBLE AND -RESISTANT STRAINS OF M. TUBERCULOSIS[1]

Relationship Between the Results of a Test for Enzymes and Those of the Indirect Drug-susceptibility Test

Catalase and peroxidase, two of the iron-porphyrin enzymes, are both normally present in the respiratory systems of mammalian strains of *Mycobacterium tuberculosis*. A relationship has been shown to exist between diminished catalase activity and resistance to isoniazid in strains of *M. tuberculosis* isolated from isoniazid-treated patients.[2] It has also been observed[3-5] that some isoniazid-resistant mutants of tubercle bacilli retain catalase activity, particularly those that are resistant to less than 10.0 γ per ml. of the drug. Therefore, the absence of catalase constitutes presumptive evidence of isoniazid resistance while the presence of catalase is not sufficiently significant to serve as a substitute for an isoniazid-susceptibility test. Recent studies [3, 6-8] have shown that catalase deficiency is not associated with resistance to streptomycin, PAS, or amithiozone.

Several investigators[4-6, 9] have found that isoniazid resistance is more closely related to peroxidase activity than to catalase activity. Deficiency in peroxidase was shown to precede reduction in catalase, and complete loss of peroxidase often occurred at very low levels of resistance. On the other hand, retention of peroxidase activity by resistant organisms has also been observed.[5] Tubercle bacilli resistant to streptomycin, PAS, or amithiozone were reported[6] to have normal peroxidase activity.

The purpose of this report is to describe the results obtained when 1,221 cultures of *M. tuberculosis* isolated from hospitalized patients were tested for isoniazid susceptibility by a conventional cultural procedure, and for catalase and peroxidase activity by the method described by Bogen.[9]

Drug-susceptibility tests were performed by the indirect (subculture) method, using Löwenstein-Jensen medium. The size of inoculum employed in this laboratory has been described elsewhere[10] and was shown to result in slightly less than confluent growth on the majority of the drug-free controls. Drug concentrations used were as follows: isoniazid, 1.0 and 5.0 γ per ml.; streptomycin, 10.0 and 100 γ per ml.; and PAS, 10.0 and 100 γ per ml. Two drug-free controls accompanied each test: one control at the beginning and one at the end of each set of drug-containing media. The inoculated slants of medium were incubated at 37°C. for four weeks before final readings were made.

For the purpose of this report, cultures that revealed no growth on medium containing 1.0 γ of isoniazid per ml., but good growth on the controls, are defined as "susceptible." Cultures that revealed less growth on medium containing either 1.0 γ per ml. or 1.0 and 5.0 γ per ml. of the drug than they revealed on the controls are referred to as "partially resistant." Cultures that yielded growth on 1.0 γ per ml., or on both 1.0 and 5.0 γ per ml. of isoniazid, equal in either instance to that on the controls, are referred to as "resistant." This method of reference was adopted because resistance to these two concentrations of the drug was so closely related. Of the total 716 cultures which revealed some degree of resistance to the 1.0 γ per ml. level, 539 (75.3 per cent) also revealed a similar, or a lesser, degree of resistance to 5.0 γ per ml. of this antimicrobial agent.

The reagent used in tests for catalase and peroxidase activities was prepared as follows:

(*a*) Hydrogen peroxide, 30 per cent (Superoxol®) (Merck) was diluted 1:30 with distilled water to obtain fresh 1 per cent hydrogen peroxide. (*b*) Sufficient catechol was dissolved in distilled water to obtain a 0.2 per cent solution. The working reagent, prepared just prior to use, consisted of a mixture of equal parts of these two solutions.

Catalase and peroxidase tests were performed by a bacteriologist who was unaware of the drug susceptibilities of the cultures being examined.

[1] This study was supported in part by funds provided under Contract No. AF 41 (657)-26 with the USAF School of Aviation Medicine, Randolph Field, Texas.

[2] Middlebrook, G.: Am. Rev. Tubere., 1954, *69*, 471.

[3] Middlebrook, G., *et al.*: Am. Rev. Tubere., 1954, *70*, 852.

[4] Steif, M., *et al.*: Tr. Seventeenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration—Armed Forces, 1958, p. 279.

[5] Hedgecock, L. W., and Fauscher, I. O.: Am. Rev. Tubere., 1957, *75*, 670.

[6] Tirunarayanan, M. O., and Vischer, W. A.: Am. Rev. Tubere., 1957, *75*, 62.

[7] Knox, R., *et al.*: Am. Rev. Tubere., 1956, *73*, 726.

[8] Van Liew, R. M.: Am. Rev. Tubere., 1957, *76*, 1007.

[9] Bogen, E.: Am. Rev. Tubere., 1957, *76*, 1110.

[10] Dunbar, F. P., *et al.*: Am. Rev. Tubere., 1958, *77*, 350.

TABLE 1

COMPARISONS BETWEEN CATALASE ACTIVITY, PEROXIDASE ACTIVITY, AND ISONIAZID SUSCEPTIBILITY IN 1,221 CULTURES OF M. TUBERCULOSIS ISOLATED FROM SPUTUM SPECIMENS

Enzyme Activity*	Susceptibility to Isoniazid						Totals	
	Susceptible (1.0 γ per Ml.)		Partially Resistant (1.0 to 5.0 γ per Ml.)		Resistant (1.0 to 5.0 γ per Ml.)			
	(*number*)	(*per cent*)	(*number*)	(*per cent*)	(*number*)	(*per cent*)	(*number*)	(*per cent*)
Catalase:								
+	483	55.6	177	20.4	209	24.0	869	100.0
+ and −	10	12.5	22	27.5	48	60.0	80	100.0
−	12	4.4	82	30.2	178	65.4	272	100.0
Peroxidase:								
+	444	80.4	59	10.7	49	8.9	552	100.0
+ and −	29	31.2	34	36.6	30	32.2	93	100.0
−	32	5.6	188	32.6	356	61.8	576	100.0

* (+) Indicates that all colonies showed activity by the enzyme noted.
(+ and −) Indicates that some colonies showed activity by the enzyme noted while others did not.
(−) Indicates that none of the colonies showed activity by the enzyme noted.

Growth on the first drug-free control of each susceptibility test was covered with the above reagent. The presence of catalase was indicated when gas bubbles were emitted by the colonies within two to five minutes. The presence of peroxidase was indicated when the colonies turned brown to black within forty-five minutes. The isoniazid-susceptible H37Rv strain of *M. tuberculosis* was used as the control organism. Results of these tests were recorded as follows: (+) when all colonies revealed activity by the enzyme noted; (+ and −) when some colonies revealed activity by the enzyme concerned while others did not; and (−) when none of the colonies showed activity by the enzyme noted.

Comparisons between catalase activity, peroxidase activity, and susceptibility to isoniazid are listed in table 1. As shown, 55.6 per cent of the 869 catalase-positive cultures were susceptible to isoniazid, 20.4 per cent were partially resistant, and 24.0 per cent were resistant. On the other hand, 80.4 per cent of the 552 peroxidase-positive cultures were susceptible to this drug, 10.7 per cent were partially resistant, and 8.9 per cent were resistant. The fact that catalase-positive colonies appeared more frequently than peroxidase-positive colonies in cultures containing resistant organisms accounts for the differences noted with respect to the activities of the two enzymes in susceptible cultures. The relationship between peroxidase activity and isoniazid susceptibility of catalase-positive cultures is shown in table 2. It may be noted that 439 (81 per cent) of the 542 catalase-positive, peroxidase-positive cultures were isoniazid susceptible, while the proportions of susceptible cultures dropped for cultures deficient in peroxidase.

TABLE 2

RELATIONSHIPS BETWEEN CATALASE AND PEROXIDASE ACTIVITIES AND SUSCEPTIBILITY TO ISONIAZID IN 869 CULTURES OF M. TUBERCULOSIS COMPOSED ENTIRELY OF CATALASE-POSITIVE COLONIES

Enzyme Activity*		Susceptibility to Isoniazid			Totals
		Susceptible	Partially Resistant	Resistant	
Catalase	Peroxidase	Number			
+	+	439	57	46	542
	+ and −	25	32	21	78
	−	19	88	142	249
Totals		483	177	209	869

* *See* footnote to table 1

The peroxidase activities and degrees of susceptibility of cultures composed of mixtures of catalase-positive and -negative colonies are listed in table 3. As illustrated in this table, the majority of these 80 cultures were deficient in one or both enzymes, and 70 of the cultures contained resistant organisms.

As shown in table 4, only 7 (2.6 per cent) of the 265 cultures in which both enzymes were absent were composed entirely of isoniazid-susceptible organisms. Stated otherwise, 97.4 per cent of the catalase-negative, peroxidase-negative cultures were either partially resistant or resistant to isoniazid. Seven of these 272 catalase-negative

TABLE 3

RELATIONSHIPS BETWEEN CATALASE AND PEROXIDASE ACTIVITIES AND SUSCEPTIBILITY TO ISONIAZID IN 80 CULTURES OF M. TUBERCULOSIS CONTAINING BOTH CATALASE-POSITIVE AND -NEGATIVE COLONIES

Enzyme Activity*		Susceptibility to Isoniazid			Totals
		Susceptible	Partially Resistant	Resistant	
Catalase	Peroxidase	Number			
+ and −	+	1	2	2	5
	+ and −	3	1	9	13
	−	6	19	37	62
Totals		10	22	48	80

* *See* footnote to table 1

TABLE 4

RELATIONSHIPS BETWEEN CATALASE AND PEROXIDASE ACTIVITIES AND SUSCEPTIBILITY TO ISONIAZID IN 272 CULTURES OF M. TUBERCULOSIS COMPOSED ENTIRELY OF CATALASE-NEGATIVE COLONIES

Enzyme Activity*		Susceptibility to Isoniazid			Total
		Susceptible	Partially Resistant	Resistant	
Catalase	Peroxidase	Number			
−	+	4	0	1	5
	+ and −	1	1	0	2
	−	7	81	177	265
Totals		12	82	178	272

* *See* footnote to table 1

cultures were composed entirely or partially of peroxidase-positive colonies. The significance of this finding is not known because, with the method of testing used, colonies were recorded as peroxidase positive purely on the basis of change in color within forty-five minutes.

In this study of 1,221 cultures, catalase activity was exhibited by the vast majority of the cultures that revealed peroxidase activity. On the other hand, peroxidase activity was not shown by a large number of cultures having catalase activity. These differences were correlated with the results of the cultural method of determining drug susceptibility, and it was found that the presence of peroxidase was more significant than the presence of catalase with respect to isoniazid-susceptible cultures. There was little difference in correlation between the results of the chemical and cultural tests when the absence of one or both enzymes was noted.

The final value of the results of the chemical test on a given culture, in relation to the chemotherapy of tuberculosis, can be assessed more accurately by the physician treating the individual patient. For example, in some instances it is necessary to know only that the majority of the organisms tested are susceptible to isoniazid.

The difference in time required to perform each of the procedures used in this study varies from less than one hour for the chemical test to three to four weeks for the indirect (subculture) drug-susceptibility test. Therefore, the chemical test for the presence or absence of catalase and peroxidase should be found useful as a rapid presumptive procedure on cultures from selected patients.

In summary: Comparisons were made between the results of catalase tests, peroxidase tests, and cultural tests (indirect) for isoniazid susceptibility. When isoniazid-susceptible cultures were considered, positive peroxidase tests correlated more closely than positive catalase tests. On the other hand, when isoniazid-resistant cultures were considered, either a negative catalase or a negative peroxidase test was found to be presumptive evidence of isoniazid resistance.

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URINE TEST FOR THE DETECTION OF PAS IN AMBULATORY TUBERCULOUS PATIENTS

Recent reports in the English literature[1] and incidental observations in the chest clinics of the Department of Health of New York City emphasize the difficulties in administering oral PAS treatment to ambulatory tuberculous patients. Patients are reluctant to take PAS treatment because of the inconvenience of taking a large number of tablets daily and because of frequent gastrointestinal reactions. To obtain some information as to the size of the problem, the following three groups of patients were examined by doing a single urine test for PAS according to the method described by Dixon and associates:[2]

A. Hospitalized tuberculous patients receiving PAS treatment

B. Tuberculous patients receiving PAS treatment in chest clinics

C. Control groups of nontuberculous persons (medical students and nontuberculous hospitalized patients)

All tests were carried out by the writer. None of the patients was aware of the reason a urine specimen was requested. The chest clinic patients on PAS treatment (Group *B*) originated from five chest clinics of the New York City Health Department and from the outpatient department of the hospital in which the inpatients were examined. Since there was no statistical difference in the results of the urine tests between the patients from Health Department clinics and from the hospital outpatient department, the results of all clinic patients were combined. A variety of PAS preparations was available to the ambulatory and hospital patients.

In 90 per cent of the hospitalized patients a positive urine test for PAS was obtained, while in ambulatory patients only 35.5 per cent of the urine tests were positive for PAS. In the control group, several positive urine tests for PAS were obtained in diabetic patients. It is realized that the number of patients examined is small and consists of a variety of patients, and that only a single test was done. Nevertheless, the results of the urine examinations imply that outpatient treatment with PAS is not satisfactory. The findings in this small study illustrate the importance of an unsolved aspect in the present-day treatment of tuberculosis; can we obtain a more suitable PAS preparation from the viewpoint of dosage and toxic reactions? If PAS is not suitable for ambulatory treatment, is isoniazid alone a satisfactory regimen for arrested tuberculous patients with a history of recent active disease, or should other drugs be recommended to replace PAS? Is there a need for a reliable test for isoniazid since the difficulty with PAS treatment in outpatients may reflect a general problem of long-term treatment with any drug?

TABLE 1

RESULTS OF URINE TESTS FOR PAS

Type of Patient	Number of Patients	Number of Patients with Urine Positive for PAS	Percentage
Group A: Hospitalized tuberculous patients...	29	26	90
Group B: Tuberculous patients in chest clinics	76	27	35.5
Group C: Control (nontuberculous patients and medical students).	77	8*	10

* Seven of these were known diabetics and the positive test was assumed to be due to acetoacetate. The remaining false-positive test could not be accounted for.

In summary: A single urine test in ambulatory tuberculous patients receiving PAS treatment showed that less than one-half of the patients actually took the drug. The need for objective tests to determine drug acceptability by outpatients is emphasized.

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November 4, 1958

[1] WYNN-WILLIAMS, N., AND ARRIS, M.: Tubercle, 1958, *39*, 138.

[2] DIXON, W. M., *et al.*: Lancet, 1957, *273*, 871.

EFFECTS OF KANAMYCIN, STREPTOVARICIN, PAROMOMYCIN, NOVOBIOCIN, AND RISTOCETIN ON MURINE LEPROSY

Kanamycin[1] has been reported to be active against experimental tuberculosis in mice[2] and guinea pigs,[3] and against murine leprosy in mice.[4] Streptovaricin and paromomycin are also active against tuberculosis in mice,[5-7] while novobiocin and ristocetin are not.[5, 9] In human tuberculosis, kanamycin reveals definite effectiveness,[10] but the effect of streptovaricin is only slight.[5]

The present report deals with studies of the effects of these five antimicrobials on murine leprosy in the mouse.

The writer's technique of three-month chemotherapeutic assay, using intraperitoneally infected mice, has been reported.[11] Weights of omenta and pelvic fatty pads, a "leprosy index" which is an average evaluation of gross lesions in various sites and organs, and a "bacillus index" representing the relative number of bacilli in smears from different sites and organs are the bases for the assay. In some experiments, bacillary counts made from standard suspensions of spleen and liver were included. A three-week chemotherapeutic assay was also employed. Microscopic grading of lesions of spreads from mesentery and bacillary counts of spleen and liver were used as criteria for estimating drug activity.

Details of techniques for study of lesions in mesenteric spreads and for counting bacilli are to be published elsewhere.[12] Briefly, the lesions in the mesentery were examined microscopically, using low-power objective and 5-mm. square grill in the ocular. Lesions were graded from 1 to 10, according to the number of infected cells per square. Bacillary counts for organs, in both three-week and three-month experiments, were made on smears prepared from suspensions. Total volume of suspensions was 4 ml. for each spleen and 7 ml. for each liver. Using a 3-mm. platinum loop, duplicate smears of 13 mm. in diameter were made from undiluted or suitably diluted suspensions. Bacillary count for each organ is the average for 200 squares (oil immersion).

Female albino mice of general purpose strain of the National Institutes of Health, weighing 16 to 20 gm., were used in groups of 10 (for three-week experiments) or 20 (for three-month experiments), each group being caged separately. Each mouse was inoculated intraperitoneally with 0.5 ml. of a 1:30 suspension in normal saline of the omenta and pelvic fatty pads from mice which had been infected four to five months previously with the Hawaiian strain of *Mycobacterium leprae murium*.

Streptomycin, isoniazid, and 4,4'-diaminodiphenyl sulfone were used as standards of reference for drug activity. Kanamycin, streptomycin, and paromomycin were injected subcutaneously, daily, five days a week. Ristocetin was injected twice daily. Streptovaricin, novobiocin, isoniazid, and the sulfone were mixed in the powdered Purina® rat chow.

Treatment was commenced on the day after inoculation. All animals were sacrificed at either three weeks or three months.

Three-week experiments: Three experiments were performed, the results of which are shown in table 1. The total leprosy scores, i.e., the leprosy score for the mesentery plus bacillary counts of spleen and liver of the three untreated leprosy control groups, ranged from 13.1 to 20.1. The total leprosy scores of five groups receiving the three reference drugs ranged from 5.3 for isoniazid in the second experiment to 11.0 for streptomycin in the second, being smaller than that for the control group in each experiment. Streptovaricin, paromomycin, and kanamycin revealed suppressive activity, as shown by their relatively small total leprosy scores, i.e., from 6.3 to 8.4. Thus the sup-

[1] Kanamycin sulfate (Kantrex®) was kindly supplied by Bristol Laboratories, Inc.; paromomycin by Parke, Davis & Co.; streptovaricin (Dalacin®) and novobiocin (Albamycin®) by The Upjohn Co.; and ristocetin (Spontin®) by Abbott Laboratories.

[2] YANAGISAWA, K., AND SATO, N.: J. Antibiot., 1957, *10*, 233.

[3] YANAGISAWA, K., *et al.*: J. Antibiot., 1957, *10*, 236.

[4] KAWAGUCHI, Y., *et al.*: Jap. J. Microbiol, 1958, *2*, 95.

[5] MCCUNE, R. M., *et al.*: Am. Rev. Tuberc., 1957, *75*, 659.

[6] RHULAND, K. F., *et al.*: Am. Rev. Tuberc., 1957, *75*, 588.

[7] FISHER, M. W.: Personal communication.

[8] WILKINS, J. R., *et al.*: Antibiotics and Chemother., 1956, *6*, 149.

[9] HUANG, K.: Personal communication.

[10] DONOMAE, I.: Ann. New York Acad. Sc., 1958, *76*, 166.

[11] CHANG, Y. T.: Internat. J. Leprosy, 1953, *21*, 47.

[12] CHANG, Y. T.: Am. Rev. Tuberc. (submitted for publication).

TABLE 1

EFFECTS OF KANAMYCIN, STREPTOVARICIN, PAROMOMYCIN, AND RISTOCETIN IN MOUSE LEPROSY
Three-week Experiments

Number of Experiment	Drug	Dose per Mouse	Leprosy Score in Mesentery	Bacillary Count		Total Leprosy Score
				Spleen	Liver	
		(*mg.*)				
1	Leprosy control, untreated		2.0	4.8	12.2	19.0
	Streptomycin	2.0	0.8	1.4	5.2	7.4
	Isoniazid*	0.01%	0.7	1.0	5.1	6.8
	Streptovaricin*	0.5%	0.6	1.3	4.5	6.4
	Paromomycin	3.0	0.9	2.0	3.8	6.7
2	Leprosy control, untreated		2.6	5.6	11.9	20.1
	Streptomycin	2.0	1.7	5.0	4.3	11.0
	Isoniazid*	0.01%	0.6	0.9	3.8	5.3
	Streptovaricin*	0.5%	1.7	2.1	4.6	8.4
3	Leprosy control, untreated		4.0	2.2	6.9	13.1
	Streptomycin	2.0	1.9	2.0	4.5	8.4
	Kanamycin	4.0	2.5	1.2	2.6	6.3
	Ristocetin	1.0 × 2	3.0	3.0	10.1	16.1

* Drug was mixed in the diet.

TABLE 2

EFFECTS OF KANAMYCIN, STREPTOVARICIN, PAROMOMYCIN, NOVOBIOCIN, AND RISTOCETIN IN MOUSE LEPROSY
Three-month Experiments

Number of Experiment	Drug	Dose per Mouse	Number Died/ Number Used	Body Weight	Weight of Omentum	Weight of Pelvic Fatty Pads	Bacillary Count		Bacillus Index	Leprosy Index	ICE*
							Spleen	Liver			
		(*mg.*)		(*gm.*)	(*gm.*)	(*gm.*)					
1	Normal mice control		0/20	23.5	0.01	0.48					
	Leprosy control, untreated		0/20	25.8	0.12	1.25			31.0	8.28	
	Streptomycin	2.0	4/20	24.6	0.05	0.75			15.5	2.02	4.1
	Paromomycin	3.0	0/20	23.7	0.10	0.92			21.5	2.86	2.9
	Novobiocin†	0.1%	3/20	23.5	0.13	0.91			26.0	8.99	0.9
2	Leprosy control, untreated		2/20	20.9	0.18	0.73	247	1,216	36.0	11.14	
	4,4′-diaminodiphenylsulfone†	0.1%	1/20	22.7	0.11	0.60	67	183	24.0	7.21	1.6
	Isoniazid†	0.01%	0/20	22.6	0.01	0.45	1	2	8.0	0.88	12.7
	Streptovaricin†	0.1%	0/20	20.7	0.11	0.43	5	31	17.0	5.09	2.2
	Streptovaricin†	0.2%	0/20	22.4	0.07	0.56	1	8	14.0	2.75	4.1
	Streptovaricin†	0.5%	1/20	24.0	0.04	0.38	1	3	10.5	1.29	8.6
3	Leprosy control, untreated		0/20	21.3	0.18	0.91	385	1,535	32.5	9.48	
	Streptomycin	2.0	2/20	22.0	0.10	0.71	92	103	16.0	3.85	2.5
	Kanamycin	4.0	0/20	23.4	0.05	0.86	7	6	9.0	1.15	8.2
	Ristocetin	1.0 × 2	0/20	18.8	0.11	0.79	589	2,035	18.0	9.80	1.0

* Index of chemotherapeutic effectiveness, *see* text.
† Drug was mixed in the diet.

pressive activities of these three drugs were similar to those of the reference drugs.

Ristocetin was inactive, its leprosy score being actually higher than that of the control. Novobiocin, in a dose of 0.1 per cent in the diet, was also inactive, as indicated by the leprosy score of the mesentery alone in an early experiment.

Three-month experiments: Three experiments were performed. Streptomycin was used as standard of reference in two experiments; the sulfone and isoniazid in one. A total of 13 animals died of undetermined causes. The results are shown in table 2.

The effect of paromomycin and novobiocin was tested in the first experiment. Streptomycin revealed marked activity, as shown by the lower weight of omentum and of pelvic fatty pads and lower bacillus and leprosy indices than those of the controls. The activity of paromomycin was slightly less than that of streptomycin. The bacillus and leprosy indices were slightly higher than those of streptomycin-treated animals, but definitely lower than those of the control animals. Novobiocin was inactive; all of its leprosy scores were of the same order as those of the control group. The index of chemotherapeutic effectiveness[13] was 4.1 for streptomycin, 2.9 for paromomycin, and 0.9 for novobiocin.

The effect of streptovaricin was studied in the second experiment. Bacillary counts of spleen and liver were included in addition to the observations made in the first experiment. Three doses were used. The small dose, 0.1 per cent in the diet, revealed activity slightly higher than that of the sulfone, as shown by smaller bacillus and leprosy indices. The index of chemotherapeutic effectiveness of the streptovaricin group was 2.2, and that of the sulfone-treated group, 1.6. Bacillary counts of spleen and liver showed marked differences between these two groups. In the streptovaricin group, the bacillary counts of spleen and liver were 5 and 31, as compared with 67 and 183, respectively, in the sulfone-treated animals. The counts in untreated controls were very high: 247 for spleen and 1,216 for liver.

The medium dose of streptovaricin, 0.2 per cent in the diet, showed greater suppressive activity than the small dose. The weight of omentum and the bacillus index were each about one-third of those for controls; and the leprosy index, about one-fourth. The large dose, 0.5 per cent in the diet, was most effective, its leprosy index approaching that of the most active drug, isoniazid. Bacillary counts of spleen and liver for animals given isoniazid and the medium and large doses of streptovaricin were very low and similar to one another. The index of chemotherapeutic effectiveness of small, medium, and large doses of streptovaricin was 2.2, 4.1, and 8.6, respectively, showing a quantitative relationship between suppressive activity and dosage of the antimicrobial.

In the third experiment, kanamycin showed greater activity than streptomycin. The weight of omentum, bacillary counts of spleen and liver, and bacillus and leprosy indices were markedly lower than those of the streptomycin-treated animals. The index of chemotherapeutic effectiveness of kanamycin was 8.2; and that of streptomycin, 2.5.

Ristocetin was not well tolerated, as shown by loss of weight of the animals. The bacillary counts of spleen and liver and the leprosy index of this group were higher than those of the control group, although weight of omentum and bacillus index were lower. This antimicrobial drug is evidently inactive in this infection.

* * *

The results obtained in the three-week experiments agree in general with those obtained in the three-month experiments. Kanamycin, streptovaricin, and paromomycin, and the three reference drugs, streptomycin, isoniazid, and the sulfone, were found active in both. Differences in activity among these agents were seen only in the longer experiments. Novobiocin and ristocetin were found inactive in both shorter and longer tests.

McCune and associates[5] reported that streptovaricin, administered for twelve weeks in a dosage of 0.2 per cent in the diet, has a slightly inhibitory effect in mouse tuberculosis. The bacillary population of spleen and lungs was very high in the early days of infection. Although a slow reduction of the microbial census in the spleen began after three weeks' treatment, the organism in the lungs remained at a high level throughout. This is quite different from the results obtained in mouse leprosy. As described above, the bacillary counts of spleen and liver were very low after three weeks and reached a minimum after three months of therapy.

Kanamycin, in a dose of 4 mg. per mouse, showed suppressing activity in mouse leprosy definitely superior to that of streptomycin in a dose of 2 mg. It is interesting to note that with the above doses these antimicrobials exhibited equal activity in mouse tuberculosis.[2] Both streptovaricin and kanamycin, therefore, appear to be more

[13] The index of chemotherapeutic effectiveness was calculated as follows: (Leprosy index of control group)/(Leprosy index of treated group). The higher the figure the higher the activity. Unity means no activity.

active in mouse leprosy than in mouse tuberculosis.

Paromomycin, in a dose of 3 mg. per mouse, showed slightly less activity than did streptomycin in a dose of 2 mg., a result parallel to that in mouse tuberculosis.[7]

Novobiocin and ristocetin were inactive in mouse leprosy, as has been found in mouse tuberculosis.[8, 9]

In summary: The effects of kanamycin, streptovaricin, paromomycin, novobiocin, and ristocetin were studied on murine leprosy in the mouse. Kanamycin, streptovaricin, and paromomycin possessed suppressive activity in both three-week and three-month experiments. Novobiocin and ristocetin showed no activity.

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[14] With the technical assistance of Robert W. Scaggs.

ELIMINATION OF PRECLEANING CAGE-LAID HENS' EGGS IN PREPARATION OF EGG FLUID FOR TUBERCULOSIS BACTERIOLOGIC MEDIA

The procedure for the handling of hens' eggs in the preparation of media for the growth of tubercle bacilli[1] has required certain time-consuming techniques to render them free of bacteria and suitable for use. It has been found during the past few years that this preliminary treatment is no longer necessary. Cage-laid eggs can be opened directly into a sterile container without first washing or soaking them in disinfectant. This egg fluid is then incorporated into the mineral base in the usual manner.

These good results, with no contamination of finished media and elimination of the cleaning procedure, can be attributed to the fact that with present-day cage-laying techniques the egg reaches the consumer in a much more sanitary condition. With nest-laying the egg remains in contact with the hen for some time, exposed to many bacteria and fungi, thus making it necessary to disinfect the egg shell. With modern cage-laying techniques, as soon as the egg is dropped it rolls away from the hen into a conveyor where it is immediately packaged and ready for the consumer. The use of insecticides in the laying house also cuts down on the bacterial flora.

To show the marked difference in bacterial count of nest-laid eggs compared to cage-laid eggs, and to demonstrate that precleaning of cage-laid eggs is no longer necessary, the following simple test was made:

Six nest-laid and 6 cage-laid eggs were allowed to remain undisturbed in measured amounts of sterile saline for one minute. A measured aliquot of the two solutions was added to pour plates of Difco plate count agar (tryptone glucose extract agar) and incubated for twenty-four hours at 37°C.

The final bacterial count for nest-laid eggs was found to be approximately 150,000 per egg, while only 3 bacterial colonies were found from the 6 cage-laid eggs examined.

In summary: When using cage-laid eggs, one may eliminate precleaning and disinfecting the outside shell before preparation of egg fluid used in media for the cultivation of tubercle bacilli.

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[1] Tuberculosis Laboratory Methods: VA-Armed Forces Cooperative Study on the Chemotherapy of Tuberculosis, June, 1958, Part II, Appendix, p. 27.

Correspondence

BCG VACCINATION IN SWEDEN

To the Editor of the American Review of Tuberculosis and Pulmonary Diseases:

I am forwarding to you a copy of a report by a committee consisting of the Swedish Association of Chest Specialists and the Swedish Medical Board. This report came to me, with permission from Dr. Gunnar Dahlstrom and Dr. J. Lundquist to have it published as a letter in the AMERICAN REVIEW OF TUBERCULOSIS if such seems advisable.

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January 19, 1959

OFFICIAL REPORT OF COMMITTEE OF THE SWEDISH ASSOCIATION OF CHEST SPECIALISTS AND SWEDISH MEDICAL BOARD, MARCH, 1958

The Board of Svenska Lünglakarforbundet (The Swedish Association of Chest Specialists) in collaboration with the Swedish Medical Board published the following statement on BCG vaccination in Sweden:

In consideration of the falling incidence of tuberculosis the question has arisen if the time might not be ripe for reduction of the BCG vaccination programme that has been developed in this country.

Since the introduction of BCG vaccination in Sweden 30 years ago and since the propaganda for mass vaccination of children and young people, the tuberculosis morbidity has greatly diminished and the mortality in early childhood has been almost completely eliminated. The decrease in childhood mortality is unquestionably largely the result of the favorable effect of the BCG vaccination. Another important factor is the lowered exposure risk. While the incidence of spontaneous tuberculin-positive reactions 25 to 30 years ago was about 50 per cent at the end of childhood, it is now probably only one-tenth of that figure. In a densely populated area such as Stockholm only about 1 per cent of the children are spontaneously positive at the time they start school.

The risk of tuberculosis infection is not quite negligible yet, however. Between 4,000 and 5,000 cases of open tuberculosis are registered at our dispensaries, and about 5,000 to 6,000 newly discovered cases of tuberculosis are registered annually. Among these newly detected cases are teachers, other school personnel, and members of other professional groups that come into contact with children and young people. Therefore, no more extensive reduction of the vaccination program should be considered yet.

The following recommendations are intended to serve as a guide for the institution of practical measures during the next few years. If the tuberculosis situation changes appreciably, the recommendations must be reconsidered within a reasonable length of time.

1. Although the occurrence of tuberculosis during the first year of life is extremely rare at present, there is nevertheless the known low resistance to the disease at that age. More than 90 per cent of those vaccinated as newborn babies are still tuberculin positive up to seven years of age, and those vaccinated in infancy certainly have a good protection for many years against the consequences of tuberculosis infection. Since BCG vaccination is also very simple to carry out in practice in our maternity departments (among other advantages, no previous tuberculin test is necessary), vaccination of healthy, full-term infants should be retained at present. Follow-up tests with tuberculin are usually necessary only on children in a tuberculous environment.

2. Primary tuberculosis in early school years is relatively benign. Those vaccinated as infants certainly have some protection as a result of this vaccination against the risks of infection that may be encountered during the course of their schooling. Therefore, it would seem that vaccination of the tuberculin-negative children starting school could be curtailed without disadvantage.

After school years, when the young people take employment, the risk of tuberculosis infection is unquestionably considerably increased, and primary tuberculosis in young adults is in itself a serious disease. Therefore, tuberculin-negative students should be vaccinated in good time before the end of their schooling. Tuberculin-negative children can expediently be vaccinated, or revaccinated, at 10 or 11 years of age, accordingly while in the fourth grade instead of during the sixth or seventh years of school as is now the rule. If the vaccination is to be done during the fourth school year, however, it must be assured that the graduating classes receive vaccination during the transitional period. Vaccination performed at that time affords good protection against the consequences of tuberculosis infection during puberty and early adulthood. Propaganda for vaccination

of tuberculin-negative school children 10 and 11 years of age is therefore well justified.

3. Vaccination of those called up for military service is an important preparedness measure. Previously it has been the responsibility of the pastorates to send enlistment cards to the dispensaries so the conscripts could be offered tuberculin tests and BCG vaccination before entering military service. The regulations covering this have since been abolished. It is the duty of the military medical staff, now as earlier, to carry out tuberculin tests and offer vaccination to regular enlisted men and conscripts who have been found to be tuberculin negative at the time they are posted or, respectively, when they first present themselves for duty. Vaccination of those who are tuberculin negative before their arrival in military camp has certain advantages, however, and it should be beneficial if voluntary cooperation between the military authorities and the dispensaries could be established to permit vaccination in conjunction with the conscription where possible.

4. Vaccination of women at 20 years of age, corresponding to that of the men during military service, cannot be carried out for practical reasons. Vaccination is therefore desirable for tuberculin-negative students in public high schools, municipal girls' high schools and grammar schools, elementary-school teachers' training colleges, etc., as proposed in the regulations of the respective educational institutions.

5. Within certain groups, e.g., seamen and medical personnel of various categories, the tuberculin-negative have long received BCG vaccination, and the vaccination of these groups should unquestionably be continued.

6. In accordance with instruction to dispensary physicians, tuberculin-negative individuals living in a tuberculous environment should be BCG-vaccinated to the greatest possible extent. This vaccinating activity still constitutes an extremely important link in the tuberculosis prophylactic work of the dispensaries. Since tuberculosis is now relatively common in higher age groups, environmental vaccinations should be carried out regardless of the age of the tuberculin-negative person.

COMMITTEE:

Dr. C. S. Backman, Physician-in-Chief, Sjo-Gunnarsbo Sanatorium, *Asunden*, Sweden; Chairman of the Swedish Association of Chest Physicians

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Dr. H. Hallander, Head of the Central Chest Clinic, *Linkoping*, Sweden

Dr. S. Jerlock, Head of the Central Chest Clinic, *Jonkoping*, Sweden

Dr. I. Källqvist, Physician-in-Chief, Lots Sanatorium, *Malmby*, Sweden

Dr. J. Lundquist, Chief of the Tuberculosis Section, Swedish Medical Board; Secretary-General of the Swedish National Association Against Tuberculosis

Professor A Wallgren, Emeritus Professor of Pediatrics, Royal Caroline Medical School, *Stockholm*, Sweden

SURGICAL REPORTING

To the Editor of the American Review of Tuberculosis and Pulmonary Diseases:

Since patients subjected to surgery are carefully selected and standards for selection vary, it is difficult to compare results from various clinics. That this is true is exemplified by the lack of agreement of many published opinions. It follows, then, that, if statistics are to be comparable, similar cases must be compared. Reports of small selected groups with many unknowns are of little value. However, if these cases can be qualified properly, valuable information may be derived.

More specifically, with regard to surgery for pulmonary tuberculosis, it would appear of relatively little use to present one hundred cases of this or that technique unless the patients are classified at least according to bacteriology, extent of disease, and drug therapy. Future experience may indicate that other factors may be important.

It is not sufficient to write generally of "drug-resistant cases." Organisms resistant to streptomycin alone can be expected to react normally to isoniazid. Patients whose sputum or resected lung contain culturable tubercle bacilli can be expected to have a higher incidence of postoperative tuberculous complications than those whose sputum or resected tissue do not. Generally, resected pulmonary tissue yields a higher incidence of culturable tubercle bacilli than the expectorations. Tubercle bacilli which are identifiable microscopically and which cannot be cultured are not nearly as dangerous as those which can be cultured.

Patients with minimal pulmonary tuberculosis can be expected to have fewer postoperative complications than those with far advanced disease, other factors being equal.

Various drugs have different potentialities. In many instances isoniazid may be a more effective antituberculous drug than viomycin. Two antituberculous drugs given simultaneously tend to

yield better results than either of them alone, especially as drug therapy is prolonged. Various details with reference to surgery are of the utmost importance. An example of this is the occurrence of postoperative pleural air spaces, as these are often precursors of so-called "reactivations." Moreover, since many details of technique escape description, it would seem that series of operations performed by one or a few surgeons would be more comparable than those performed by many surgeons from different institutions. It would also seem desirable that statistics be gathered and analyzed by the most experienced surgeon rather than, as commonly occurs, passing the "scut work" on to the most inexperienced, since it is obvious that proper evaluation requires a great deal of clinical experience. Furthermore, complications should be clearly defined, especially with respect to bacteriology. This would tend to obviate attributing nontuberculous complications to tuberculosis, for there are many complications such as empyema and bronchopleural fistula which can occur following resection for nontuberculous disease.

David V. Pecora

Ray Brook State Tuberculosis Hospital
Ray Brook, New York

January 9, 1959

Rehabilitation and Occupational Therapy in Tuberculosis Hospitals

To the Editor of the American Review of Tuberculosis and Pulmonary Diseases:

In the October issue of the American Review of Tuberculosis the editorial staff invited comments regarding rehabilitation and occupational therapy in tuberculosis hospitals, and I am very happy to avail myself of this opportunity to summarize my opinions briefly as they were published in other articles.

Back in the 1930's, when the current concept of rehabilitation was formulated, it was felt that physical exertion was the main cause of reactivation and therefore the patient should be re-trained to do a job requiring as little activity as possible. At that time the average age of the tuberculous patient was around twenty years. For the most part these patients had no trade or profession and needed any educational facilities that could be given them. In sharp contrast to this, today's patients average over forty years of age, and most of them have already learned and profited by a trade or profession. Those in this age group whose means of livelihood has been manual labor probably could not be taught a trade. A significant percentage of our patients come from the low economic level, many cannot read or write, and for these people rehabilitation to a lighter job is impossible. By ordering them to do no manual labor they automatically become recipients of welfare for the rest of their lives. In my experience, patients who could be re-trained spent at least a year being rehabilitated, then took jobs that paid less than their original positions, and before many months were back at their old jobs out of economic necessity. Physical exertion per se is no factor in the reactivation of tuberculous patients after drug therapy, so the original premise does not apply today. Reactivation is due to just one thing: the presence of viable, virulent tubercle bacilli in tissue into which the drugs cannot penetrate, and therefore it is the physician's responsibility either to rule out the presence of such tissue or remove it.

Another phase of the accepted program was occupational therapy, and its purpose was to keep the patients busy so they would not leave the institution before treatment was completed. When this concept was originated, the average hospital stay was eighteen months or longer. In Washington State the period of hospitalization still averages seven months, but at the Central Washington Tuberculosis Hospital in Selah it was cut to four months. I have never approved of occupational therapy. It seems to me that both the therapist and the patient tend to forget that the purpose of hospitalization is treatment. Too often patients are kept busy making worthless items that even they are ashamed of. If it is true that an elaborate occupational therapy facility decreases the percentage of irregular discharges, then you would expect that an institution with no such facilities would have the highest rate of irregular discharges. I found the exact opposite to be true. Since starting a definite treatment program in 1952, only 8 per cent left against medical advice. This is less than half the percentage of irregular discharges reported by the institution in this state with the most complete occupational therapy program.

It would seem that the taxpayer is getting a very poor return from the dollars spent on rehabilitation and occupational therapy in tuberculosis institutions.

Albert R. Allen

P. O. Box 327
Selah, Washington

January 15, 1959

SOPHISTRY IN THE USE OF THE WORD "MINIMAL"

To the Editor of the American Review of Tuberculosis and Pulmonary Diseases:

The writer wishes to challenge the correctness of the cognomen "minimal" when referring to the extent of involvement with pulmonary tuberculosis. Psychologically, the word leads too often to complacency and to satisfaction on the part of the physician and in the mind of the patient.

"Minimal" means "the least amount possible or admissible," "soupçon," "small quantity," "modicum," "whit," "touch," and paints a picture of being inconsequential, equivocal, and without danger.

We portray (figure 1) so-called minimal disease of the year 1952. This little area of dynamite, this "dud?" has led to the involvement shown, in 1958, by figure 2 in a thirty-seven year old father with a large dependent family (total destruction of the left lung and involvement of the right lobe).

It is submitted that serious thought should be given to replacement of the word "minimal" by some thought- and action-provoking word such as "significant," "substantial," "early," "important," "evident" "notable," "prominent," "material," "nuclear," "consequential," or "nidal."

Knowing full well the trials and tribulations, the successes and the failures associated especially in the surgical treatment of patients with bilaterally far advanced and cavitary disease, with sputum persistently "positive," with advancing physiologic age, and with complicating factors, we contend that all of us must be ever vigilant. We assert that to patients of varying literacy and to physicians and surgeons—neophyte and master—the label "minimal" invites indifference and nonchalance. We cannot permit the power of a word to obstruct our path.

"*Ab inconvenienti*"—(legal) an argument from inconvenience or hardship has great weight.

JAMES B. SEAMAN

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East Texas Tuberculosis Hospital
Tyler, Texas

January 23, 1959

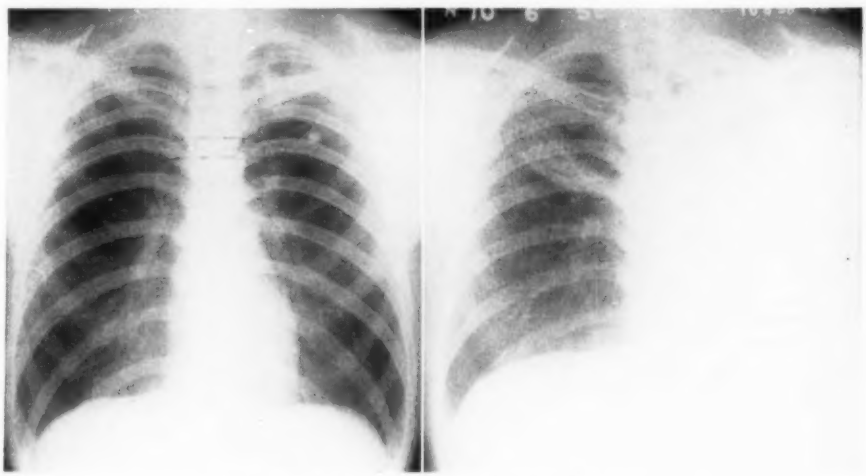

FIG. 1 (Left). "Minimal" disease of the left apex.

FIG. 2 (Right). Total destruction of the left lung with disease of the right upper lobe. Left pneumonectomy is contemplated. The right lung is to be observed; additional surgery may be necessary.

Books

DIE ENTWICKLUNG DER TUBERKULOSEBEHANDLUNG SEIT 100 JAHREN. By G. Domagk, L. Heilmeyer, and H. Kraus. Pp. 47, Georg Thieme Verlag, Stuttgart, 1958. Price, $2.85

The little brochure under review contains a number of speeches given on February 10, 1958, when the new tuberculosis research hospital of the University of Freiburg was opened. As is usual on such occasions, two-thirds of that said could have been left out without seriously impairing the importance of the event. The reviewer advises everybody who should ever open this booklet to skip the first nineteen pages. The only fact he might learn is that the new hospital is a well-built and well-equipped place offering opportunities to study tuberculosis both at the bedside and in the laboratory. The building price of 24,000 German marks per bed indicates that hospital administrators and city planners have to fight with rapidly rising costs not only on this side of the Atlantic.

A physician interested in the history of medicine will find some new information in the lecture given by Dr. Heilmeyer, chairman of the Department of Medicine, which is devoted to the memory of two pioneers in the field of bed-rest treatment, G. A. Brehmer and P. Dettweiler. A facsimile of the frontispiece of Brehmer's thesis written in 1853 demonstrates that he was one of the first European physicians to state categorically: "Tuberculosis primis in stadiis semper sanabilis" (Tuberculosis in the first stage can always be cured). The following lecture given by Nobel Prize winner Dr. Domagk deals with the problems of drug resistance and makes a strong stand in favor of the isonicotinic acid derivatives as primary means of treatment. In the author's opinion, there exists good hope that the problem of resistance against tuberculostatic drugs can be dealt with effectively. The talks following Dr. Domagk's can hardly be expected to arouse an American reader's interest with the exception, perhaps, of Dr. Griesbach's remarks, which contain the interesting statement that the death rate for tuberculosis has now reached an all-time low in West Germany with 2 cases per 10,000 deaths. The annual infection rate, however, is still rather high with 22 per 10,000, i.e., there are at present 115,000 patients with known open tuberculosis in West Germany.

DIETHELM BÖHME
New York, New York

TUBERCULOSIS IN ANIMALS AND MAN. A Study in Comparative Pathology. By John Francis. Pp. 357, Williams and Wilkins Company, Baltimore, 1958. Price, $18.00.

This book, comprised of three principal sections, will be of considerable value to all students of tuberculosis, whether their primary interest be clinical (human or veterinary), laboratory, or general ecologic.

The first section of 7 chapters is concerned with bovine tuberculosis. It embraces the epidemiology, pathology, pathogenesis, detection, immunization, and other control measures of tuberculosis in cattle. A separate chapter is devoted to bovine infection in man.

The second section contains excellent information concerning tuberculosis in animals other than bovines. This section will be of particular value to readers interested in epidemiology and laboratory investigations. It contains much information on such diverse subjects as tuberculosis in cold-blooded animals and the isolation of the vole bacillus by Dr. A. Q. Wells.

Each of the initial two sections have excellent, well-organized summaries.

The third section contains the comparison of pathology and epidemiology of tuberculosis in animals with that observed in man. Attention is given to the questions of allergy, the virulence of tubercle bacilli in various hosts, the basis of species resistance to tubercle bacilli, and the natural infection of various species with tubercle bacilli.

The reports of the British Royal Commission on Tuberculosis are given as an appendix.

More than 900 references to the world literature on tuberculosis are cited. They are split up into the following two groups: bovine tuberculosis and tuberculosis in other animals and comparative pathology.

There is a well-prepared index. The tables and photomicrographs are of commendable quality.

HERMAN R. HUEMPFNER
New York, New York

BOOKS RECEIVED

REHABILITATION OF THE CARDIOVASCULAR PATIENT. By Paul Dudley White, Howard A. Rusk, Philip R. Lee, and Bryan Williams. Pp. 176, McGraw-Hill Book Company, Inc., New York, 1958. Price, $7.00.

THE PNEUMOCONIOSIS PROBLEM, WITH EMPHASIS ON THE ROLE OF THE RADIOLOGIST. By Eugene P. Pendergrass. Pp. 146, Charles C Thomas, Springfield, Illinois, 1959. Price, $6.75.

MENSEN VAN BERG EN BOSCH. By Martien Coppens. Pp. approximately 140, Corns Wm Geleedts te Krimpen aan den IJssel, Nederlands, 1958.

CARDIOVASCULAR REHABILITATION. By Paul Dudley White, Howard A. Rusk, Bryan Williams, and Philip R. Lee. Pp. 155, McGraw-Hill Book Company, Inc., New York, 1957. Price, $6.50.

COMMUNICATIONS DES INVITES ETRANGERS. CONGRES NATIONAL DES SCIENCES MEDICALES. Pp. 908, Editions de L'Academie de la Republique Populaire Roumaine, Bucarest, 1957.

BRONCHOGRAPHY. By C. Dijkstra. Pp. 157, Van Gorcum & Comp. N.V., Assen, The Netherlands, 1959. Price, Hfl. 19.50.

American Trudeau Society

Medical Section of the National Tuberculosis Association

"Tuberculosis: A World-Wide Problem" was the subject of a conference held November 18, 1958, under the auspices of the Tuberculosis and Health Associations of Greater New York. The Council of the Tuberculosis and Health Associations of Greater New York represents the three tuberculosis and health associations of Brooklyn, New York, and Queensborough. Participating in the conference, held at the Hotel Biltmore in New York City, were Dr. Leona Baumgartner, Commissioner of the New York City Department of Health, Dr. Georges J. Canetti, Chief of Laboratories of the Tuberculosis Department of the Pasteur Institute in Paris; and Dr. Johannes Holm, Chief of the Tuberculosis Section of the World Health Organization in Geneva. Dr. James E. Perkins, Managing Director of the National Tuberculosis Association, presided at the conference, which attracted an audience of approximately 500 persons.

CHANGES IN TUBERCULOSIS AS SEEN BY A PATHOLOGIST

GEORGES J. CANETTI

I greatly appreciate the honor of having been invited to address this meeting. Some outstanding work in the fight against tuberculosis has been done in New York City; I think only of the great work done at the Rockefeller Institute, Cornell, Bellevue Hospital, and other places. No doubt this great surge in the study of tuberculosis will continue. I think it is always interesting to speak about things with which one has had a close link. My main interest for the past twenty years has been in the mechanisms of tuberculous disease. How does the tubercle bacillus produce lesions in man? What are the main factors involved?

It happens that in the past twelve years great changes have occurred in tuberculosis. Efficient chemotherapy has come; drugs active against the tubercle bacillus have been discovered, and the entire outlook of tuberculosis has changed. When I was asked to address you I was very uncertain about the choice of the subject and then I found my answer. Why not tell you about what has changed in the mechanism of tuberculous infection since chemotherapy? So this will be the main topic of my talk.

In order to understand exactly the changes which have occurred, we must first try to understand the nature of the tuberculous focus before the arrival of chemotherapy. A bacillus is inhaled; what is to happen? Three potentialities exist which lead to entirely different issues. In some happy cases no fixation or multiplication of the inhaled bacillus occurs; the bacillus is either killed by the defense mechanism of the organism or even eliminated without producing the slightest change. More often the bacillus fixes itself somewhere in the lung. It is opposed by the defensive weapons of the body, but starts multiplying. When a certain amount of multiplication is reached, the bacilli destroy a little area of lung tissue and thus create the ordinary primary focus of the lung. But then a sudden change occurs in the destroyed tissue. The bacilli no longer find the food and oxygen supply required for their life and they stop multiplying. Either all of the bacilli die or the lesion cures spontaneously, as happens in the great majority of cases. In some other cases most of the bacilli die, but not all; a few survive and these survivors may be the cause of disease many, many years later.

What we have seen up to now is an outline of latent tuberculous infection. We now come to tuberculous disease. An entirely different succession of events occurs here. The bacillus enters the body, it multiplies just as before, and destroys a little area of lung tissue. But instead of dying out in the destroyed tissue, or instead of showing no further multiplication, the increase of bacillary numbers goes on and a fundamental change occurs in the tissue which has been destroyed; it softens. The softened tissue finds its way into a bronchus and is partially eliminated; a cavity appears. One may imagine that this elimination of dead tissue, of tissue killed by the bacillus, is a good solution, a sort of cleansing of the diseased area. In fact, it is not. The dead and softened tissue is only partly eliminated. In the remaining part conditions of bacillary multiplication still improve and the situation is even worse than before. Why? The tubercle bacillus is a microbe which needs good oxygen supply for multiplication. The better the supply, the greater the extent of multiplication. The partial elimination of the dead tissue by a bronchus ensures the inlet of oxygen into the newly created cavity and provides an unhoped-

for oxygen supply for the bacillus. Its multiplication thus increases more and more and the bacilli may eventually reach tremendous numbers. For instance, in a cavity of medium size, a number close to one billion bacilli may be found. This is a number of the same range as that which would exist in an artificial medium of the same size, created just for culturing bacilli in an incubator. And not only has the elimination of the dead, softened tissue created this dramatic biologic constellation for bacillary multiplication, but it has given the bacilli the best opportunity to spread into other parts of the lung. They are in liquid particles, an open bronchus provides the way out, so they spread into new areas of the lung and produce there new disease. They spread out of the body and infect healthy people. This then is the main biologic fact in pulmonary tuberculosis: a lesion exists in which the conditions are such that the bacilli are undergoing indefinite multiplication and from which the bacilli disseminate easily.

What sort of treatment could be opposed to this situation before chemotherapy? The main treatments existed: rest and collapse therapy (pneumothorax, pneumoperitoneum, thoracoplasty). The ways by which these treatments were operative are complicated and too detailed to be fully described on this occasion. One way, for instance, was setting up mechanisms leading, eventually, to bronchial closure and thus cutting down the oxygen supply by which the bacillary multiplication had reached such high levels. These treatments were difficult and expensive. They were efficient in many cases but, by far, not in all. To many people these treatments were not available because they could not be provided without highly technical and expensive medical equipment, which many countries still lack.

Chemotherapy has introduced a fundamental change into this situation. We now have powerful drugs at our disposal which are capable of killing the tubercle bacillus. You all know the names of these drugs, mainly isoniazid, but also streptomycin, PAS, and all of the other drugs which have been added during the past years. Such drugs, some injected, some taken orally, do penetrate into the cavity. They kill the bacilli which exist there in tremendous numbers and thus make healing of the cavity possible. The cavity heals in two ways. Either it remains as a cavity but deprived of any bacterial life, a sort of dead space of no more danger to the patient; or it shrinks and changes into a scar. Tuberculosis is then cured in the great majority of cases; all of the bacilli have been killed and no relapse will occur in the future.

What I have given is rather an idyllic picture. If our drugs were really as efficient as that, if really they did kill the tubercle bacillus in the body so easily, why is tuberculosis not cured in a few days or a few weeks, just as the great majority of other infections are? For instance, syphilis, which has so often been compared with tuberculosis, may nowadays be cured by one great single injection of penicillin. We all know that such a therapeutic situation does not exist in tuberculosis. Drugs have to be given for many, many months in order to ensure healing without major risk of relapse. Why this great difference?

One of the main factors is that our drugs only act on bacilli which are in process of multiplying. In the cavity, although the numbers of bacilli are very high and the biologic conditions especially favorable, all of the bacilli are not in process of multiplying. Some are sleeping. They seem deprived of any major biologic activity, and the strange thing is that these sleepers, these persisters, as they have been called, escape the action of drugs. But these sleeping bacilli may awake and then start multiplying at that moment. If the patient is still under chemotherapy, the drugs will attack the waking bacilli and kill them. Thus, one of the major reasons why chemotherapy must be given for a long time is, probably, to provide the presence of drug at the very moment the sleeping bacilli awake or come back to life. This is one of the great difficulties in the treatment of tuberculosis, and this is probably the major reason why very short chemotherapy, although it quickly kills great numbers of bacilli, involves probably great risk of relapse.

Another difficulty is that in every great bacillary population, such as that of cavities, a few bacilli exist which are congenitally resistant to each drug, for instance, isoniazid. These bacilli are different from the persisters; they are not sleepers. Even if they multiply, they are not harmed by the drug to which they are congenitally resistant. Fortunately those bacilli are always susceptible to another drug so that by giving the patient two drugs these congenitally resistant bacilli are killed eventually, in the great majority of cases. But they are not in all, for different reasons which cannot be elaborated here, and this adds another difficulty to the treatment of tuberculosis.

To summarize, while the drugs have radically changed the perspective of biologic cure of tuberculosis, they provide by no means—at least nowadays—a weapon which makes treatment of tuberculosis comparable with that of other infectious diseases. The situation is still far more complicated, and the cure of any given case of tuberculosis still requires far more time, money, and care than that of almost all other infectious diseases.

What is the outlook for the future? The outlook is favorable. As a great proportion of the bacillary population of a case of tuberculosis is killed in a short time, there are, statistically speaking, less and less bacilli available for dissemination of the disease, and this must lead to a steady decline in the number of new cases of tuberculosis arising in a given community. However, the trend downwards is far less rapid than might be expected. Why? The main reason is that, at least in great cities, a great proportion of people, especially in older age groups, are already infected by the tubercle bacillus. They were infected many years ago, when almost everbody in great cities used to be infected at least once, and often several times, in the course of life. These people harbor the small foci referred to earlier. In some of them the bacilli have died out, but in others they are still there. In some of these people, under conditions which are to a great extent unpredictable, these bacilli, few as they are, will one day start multiplying again and produce real, clinical tuberculous disease with cavities. With our present drugs probably nothing can be done to avoid these future cases of tuberculosis, because the bacilli involved are, for the moment, sleepers, and our present drugs do not work against them. Of course, these future cases of tuberculosis could probably be avoided if all of these people were given, for the rest of their lives, antituberculous drugs in the hope of attacking any sleeping bacilli as soon as they awake, but such an over-prophylactic attitude is out of the question. So, in any great community a source of future cases exists which can hardly be suppressed by prophylactic measures; it has to dry up by the eventual disappearance of the last carriers of bacilli, and this will take a long time.

The greatest problem which remains to be solved in the near future is the fight against tuberculosis in under-developed countries. Many of these countries, for instance, India, China, the Arab countries, parts of Africa, parts of South and Central America, still have very high tuberculosis morbidity rates. This can be explained by their poor economic and sociologic conditions. These countries are medically under-equipped; the number of beds available for the treatment of tuberculous patients is so low that a really effective fight against tuberculosis, based on our Western methods, using hospital and sanatorium care for the tuberculous patient, is out of the question. What then can be done?

Pilot studies, organized by WHO and other organizations, are under way in order to find whether chemotherapy given on an ambulatory basis to patients in such countries may prove an effective solution. The final results of these pilot studies are not yet available. Preliminary information, however, indicates that ambulatory chemotherapy *is*, in all likelihood, the solution for such countries, provided the chemotherapy given is adequate. But this evidence, once established, will not solve the concrete problem. How should the numerous tuberculous patients be detected? How should they be induced to take, during many, many months, the drugs they need? Who will pay for the drugs? What about the patients who live in remote places and cannot be kept under close medical supervision? What should be done for people living with the tuberculous patient? Should they be protected by vaccination, by chemoprophylaxis, by both?

The problems which arise are numerous, and the answer may vary according to the geographic, economic, and sociologic conditions prevailing in the different countries. It will take many years before the solutions have been worked out. I want to emphasize that part of this work, painstaking and expensive as it will be, can only be done in the hospitals and the laboratories of our Western countries, with our great research traditions and facilities. For instance, we have at our disposal several antibacillary drugs but very few can be used for ambulatory chemotherapy in under-developed countries. Some are too toxic to be taken without close medical control; many are too expensive; some are of limited effectiveness. We need new efficient drugs which can be administered orally, are not toxic, and, especially, are cheap. What we need even more is a preparation of drugs, present or future ones, which would prove effective although administered on a very intermittent basis, i.e., drugs with retarded action, prepared in a special form or vehicle by which absorption in the body is protracted for several days or even weeks. Such a solution has been recently worked out for leprosy; why should not one exist for tuberculosis?

I have only outlined some of the problems which still face us in the field of tuberculosis. Our responsibilities are great. The progress to date has been tremendous. Let's try to meet with the same efficiency the situation which still challenges us.

URBAN RESERVOIRS OF TUBERCULOSIS

LEONA BAUMGARTNER

The course of tuberculosis in urban centers can be divided historically into three stages. The first, the stage of generalization, is characterized by the almost universal prevalence of tuberculous infection. Data obtained from tuberculin test surveys and clinical and pathologic examinations (fragmentary as they may be) indicate that the overwhelming majority of individuals have been infected before they reach late adolescence. The number of new cases reaches its peak in the twenties and thirties, particularly in the female, and then declines rapidly. The age curve of mortality from tuberculosis, which parallels that of morbidity, is at its highest point in the third decade of life. During the first stage tuberculosis is a disease of young men and young women.

The basic pattern of tuberculosis is not altered by the variations in infection, morbidity, and mortality rates which exist in this period. A high proportion of the populace is infected early in so-called "good" as well as "bad" neighborhoods. Clinical tuberculosis makes its appearance among the rich as well as the poor, and tuberculosis ranks high as a cause of death in all sections of the city and all occupational groups.

Our efforts at the control of tuberculosis in the stage of generalization are largely governed by its widespread prevalence. The attack is a general one, unrestricted as to place or population. Attempts are made to check the spread of infection through concentration on the media of its transmission, be they air, milk, food, clothing, or the surfaces of the walls and floors. Disinfection and fumigation are widely practiced. Emphasis is placed on case finding in the office of the private practitioner, in the clinic, and in the hospital. A campaign of health education is prosecuted vigorously through lectures, printed material, and other mass media in every walk of life and section of the city.

Primarily as a result of these measures, and improvement in socio-economic conditions, the next stage, the stage of localization, is reached. Tuberculosis is no longer generally prevalent but is now largely confined to certain specific groups. In younger age groups the level of tuberculous infection is low. Older people, the survivors of an earlier period when the disease was widespread, usually still exhibit a positive reaction to the tuberculin test. New infections occur relatively infrequently and then only under special circumstances. These fall predominantly into two categories, which are often closely related. One is the result of the spread of tuberculosis from one household member to another, usually in the same family. The other is under the crowded conditions characteristic of low socio-economic areas, where the presence of individuals with tuberculosis in communicable form makes the dissemination of infection inevitable.

These changes in tuberculous infection markedly alter the general age-specific curves of tuberculosis morbidity and mortality. The number of newly reported cases of tuberculosis in young individuals, which follow closely on conversion of the tuberculin reaction, falls sharply. The peak of the morbidity curve in the twenty- and thirty-year age group, characteristic of the stage of generalization, is obliterated. The reduction in morbidity, however, is not equal for all groups and in all sections of the city. In the crowded slum, often largely inhabited by minority groups and recent migrants from more heavily infected areas, tuberculosis remains an important problem of youth. This is also true in households anywhere which include individuals with tuberculosis in communicable form among their members.

The elderly, on the other hand, are not appreciably affected by the lower incidence of tuberculous infection in the community. Most older people had their first contact with the tubercle bacillus in their teens and have been reactors to the tuberculin test ever since. They represent the survivors of the stage of generalization which prevailed in large cities during the early years of this century. The development of active tuberculosis in this group, and particularly in its male members, is due to the low level of resistance attendant on their way of living and, possibly, to undefined physiologic stresses related to age. Since these are factors which change very slowly, no reduction in tuberculosis morbidity comparable to that in youth is noted in older age groups. In fact, though the total number of newly reported cases of tuberculosis may remain stationary or decline, the proportion occurring in individuals over forty-five increases steadily. As a result, the highest morbidity rate during the stage of localization is found beyond age sixty-five, even in cities with large populations belonging to minority groups, many of whom have recently arrived. A similar trend is found in the age distribution of deaths from tuberculosis, the peak mortality rate occurring at the very extreme of life.

At this stage selective application of measures for the control of tuberculosis rather than the general approach of earlier years is required. Older persons, who represent the major urban reservoir of tuberculosis, must receive the highest priority. Case finding must be concentrated in the locations where older men are to be found. Fortunately, these can readily be identified in most large cities, and include shelters, rooming houses, jails, and inpatient and outpatient services of municipal hospitals. Routine chest X-ray examinations must be made available to all of these facilities.

Once detected, tuberculosis must be treated promptly and vigorously in this group, for the instability of its members is well recognized. Early institutional care, provided by compulsory methods, if necessary, and carried on to the stage of noninfectiousness, is essential. Successful hospital treatment must be followed by long intervals of clinic supervision to guard against relapse. Those patients in whom therapy fails to produce or maintain arrest of their disease will require long-term institutional care in a hospital or other facility designed for this purpose. Most important, the living standards of these older men, many of whom are living a marginal existence, must be raised to a level where tuberculosis is less apt not only to reactivate but even to develop in the first place.

As indicated previously, new tuberculous infections usually manifest themselves in younger individuals whose contact with the tubercle bacillus originates in their own household or the neighborhood in which they live or work. The tuberculin test finds its real usefulness as a case-finding method in the identification of this group. It should be used extensively in low economic areas, starting with the high school population, and the reactors should receive both initial X-ray examination and periodic follow-up. When tuberculin testing is not feasible, the mass X-ray technique should be employed directly, but such surveys should be confined to sections in which tuberculosis is prevalent. Every effort should be made to discover the source of each new infection. BCG and isoniazid offer promise in the prevention of these fresh cases, and immediate treatment in a modern hospital is the best guarantee for recovery.

The last stage, the stage of individualization, has not yet been reached in any large city. Its realization is dependent on the exhaustion of the urban reservoirs of tuberculosis. This can occur only when the infected older generations have passed through their life span, and the congested population centers which breed new infections have been eliminated or controlled. At that time tuberculosis will manifest itself as an isolated phenomenon, an individual case, or small outbreak. These incidents may happen at any age and will usually be related to recent exposure to the tubercle bacillus. Epidemiologic investigation of each such occurrence should ensure prompt detection of the source of the infection. Control measures on an individual basis can then be applied with every prospect of success. In carrying these out, the principal responsibility will fall on the private practitioner. He will need assistance in the form of consultation facilities, and this is best furnished by a health department with years of experience with this disease. A public health agency can also be expected to continue to provide service in those areas and for groups not under private care. Its primary function, however, will be in a standby capacity, ready to act should situations develop which show signs of getting out of control. As Frost has so brilliantly demonstrated, when this point is reached and cases of tuberculosis do not reproduce themselves, the eradication of the disease will be in sight.

How do these considerations affect New York City, and where does it now stand in terms of the historic perspective? To the extent that the number of deaths, newly reported cases, tuberculin reactors, and other figures permit one to judge, this city has emerged from the stage of generalization and has entered the stage of localization of its tuberculosis problem. In the early part of this century tuberculosis was widely prevalent in New York. More than 30,000 cases and 10,000 deaths from tuberculosis were reported among a total population of less than five million in the year 1910. Not until the 1930's did the number of new cases annually fall below 10,000; and as late as 1938 almost half of the high school population were reactors to the intermediate-strength intradermal tuberculin test. During this period our program had as its primary objective the control of the spread of infection. It emphasized sanitary supervision in the home through visiting by public health nurses, X-ray examination of the entire adult population, increasing use of tuberculosis hospital beds, and the general health education of the public.

The close of World War II brought with it an era of unparalleled prosperity and saw the development of specific antimicrobial therapy for tuberculosis. The general application of the latter to patients with active tuberculosis in and outside of hospitals led to a precipitous decline in the frequency of tuberculous infection. By 1953 the tuberculin reaction rate in high schools, which had been more than 45 per cent in 1938, was down to less than 15 per cent. The general morbidity

rate in 1957 was one-ninth, and the mortality rate one-thirteenth, of the rates for 1910.

The decline was not uniform, however, and led to wide disparities in the extent of tuberculosis in different sections of the city. The tuberculin reaction rate was found to be ten times as high in the schools of some districts as in others. The number of new cases reported in 1957 in the Lower East Side District was over 900; in the Gravesend District with a larger population, only 66. Death rates by health center district ranged from 46 to 2.6 per 100,000 population during the same year.

These geographic differences in the distribution of tuberculosis can be closely correlated with the socio-economic characteristics of the areas represented, and their age, sex, and racial composition. At one extreme we find the shabby hotels and rooming houses of lower Manhattan with a preponderance of elderly men, and the crowded tenements of upper Manhattan and lower Brooklyn and the Bronx occupied by young Negro and Puerto Rican families. In both situations tuberculosis is commonplace, resulting from the breakdown of long-standing infections or the acquisition of new ones. At the other extreme are the prosperous middle class neighborhoods of one- and two-family houses in which tuberculosis has become so rare as to almost constitute an exotic disease.

Long-range plans or predictions in a disease which is changing as rapidly as tuberculosis are difficult. However, continuation of certain present trends can be anticipated.

The current thinking and program of the New York City Department of Health are based on a clear recognition of the facts as they exist today and the further fact that change in this field is rapid and constant. The activities in the Department have changed significantly in recent years.

In New York City, tuberculosis is now a sharply localized problem and our attack is being concentrated on local reservoirs of infection. Preventive measures like BCG and the prophylactic use of isoniazid must be and are being applied to contacts in such reservoir areas where the risk of infection is great. Emphasis is being placed on the routine X-ray examination of all hospital patients, on those getting public relief, and on the residents of selected areas where we believe our work will be most productive.

Facilities for treatment and isolation are being concentrated where they appear to be most needed. In providing these, we try to strike a proper balance between short-term and long-term care, and inpatient and outpatient service. Constant emphasis is being placed on all aspects of social help to the tuberculous and their families. This includes attention to housing and nutrition, to help in rehabilitation, and to other social welfare services for the tuberculous and for their families. More and more we view the handling of the tuberculosis problem as involving not only the patient, but also his family and the relationship of the patient and family to the community.

Official agencies have always provided the bulk of services for the underprivileged groups with which we are now especially concerned and in which the bulk of the cases are now found. This trend toward giving most help through official agencies will undoubtedly be intensified and calls for close cooperation among all. Proper handling in the future will undoubtedly involve even closer cooperation. Cooperation must, of course, be based on intelligent understanding by all of the participating agencies of the ever-changing nature of the total tuberculosis problem.

We have found that the Interdepartmental Health Council, which represents the Departments of Health, Hospitals, Welfare, and the Community Mental Health Board, is a good mechanism for establishing and maintaining such working, fruitful cooperation.

One may well ask: "Is there a place for voluntary bodies like the tuberculosis associations at this stage of tuberculosis control and will they have a role in the future?" The answer is unequivocal: "Yes, of course, if the goal of control of tuberculosis is to be achieved."

The tuberculosis associations can still play their traditional role in health education and the interpretation to the public of the meaning of the tuberculosis problem. Now and in the future, they must help to establish pilot studies and trial programs in different areas. There are indications that the associations are willing and anxious to do exactly these things.

The changing focus in tuberculosis, with its emphasis on localized reservoirs of disease, brings the tuberculosis association and the official agency even closer together than in the past. Most important, the transition from the stage of localization to that of individualization offers a new challenge to groups like those sponsoring this meeting. The spread of tuberculosis on an individual basis and the increasing importance of the private practitioner in its control call for the development of new techniques and approaches to both public and profession.

Experimentation and imaginative thinking are still needed. The voluntary agency, by its nature, can often be more venturesome, more imaginative, than are official agencies or private practitioners. Opportunity for leadership is there. I have faith that such opportunities will be embraced.

HOW CAN ELIMINATION OF TUBERCULOSIS AS A PUBLIC HEALTH PROBLEM BE ACHIEVED?

JOHANNES HOLM

Many people seem to believe that WHO was established mainly to assist the so-called underdeveloped countries in improving their health services so that they can effectively control diseases. To give assistance to these countries is definitely one of the functions of WHO, but WHO has other equally important functions which are also of interest to the most highly developed countries. I have chosen to speak about the elimination of tuberculosis as a public health problem because this might give me an opportunity to demonstrate to you, by means of practical examples, that WHO has a task to fulfill in the international health field which concerns the highly developed countries and that even for your own country, the United States, the work of WHO is not without interest.

In order fully to understand the work to which I am going to refer, it must be realized that, in the fields of public health and disease control, knowledge and experience gained in one country often is of great importance to others faced with the same problems. A condition for the useful exchange of information across the borders is, however, that the knowledge and experience is described in terms that are generally understood. There is, therefore, a need for an internationally accepted terminology, with terms that can easily be translated from one language to another. Furthermore, in order that data from different countries may be compared more or less directly, there is a need for internationally accepted standards for diagnostic and therapeutic products, for methods and techniques, and for classification. The establishment of such standards is exactly one of the constitutional functions of WHO.

All this can best be illustrated by an example which may be of special interest to the tuberculosis associations, in view of their slogan "Are You Positive?"

Tuberculin testing forms an integral part of many of the procedures in our tuberculosis program: it is used for diagnosis, for selecting persons for BCG vaccination, for tuberculosis surveys, and—a subject to which I shall return shortly—for the assessment of the effect of our tuberculosis program. Now, international exchange of data on tuberculin testing will be of value only when the tuberculins used have been standardized against a common standard and uniform methods have been employed in the testing (including dosage), in the reading of the test, and in the reporting of results.

WHO has, through its Expert Committee on Biological Standardization, established an international standard for tuberculin (one for Old Tuberculin and one for PPD) which makes it possible to express the doses of tuberculin in international units. This way of expressing doses of tuberculin is now widely accepted. It is, however, often misleading, because many of the tuberculins used today have not been standardized against the international standard by a biologic assay, which would justify expressing the doses of the products in international units. Such a biologic assay, which must include tuberculin testing of a large number of human subjects with different levels of tuberculin sensitivity, is a difficult and expensive undertaking. In order to improve this situation and to avoid the difficulties resulting from the use of tuberculins of different strengths, WHO has taken the initiative in arranging for the preparation of a large batch of PPD, sufficient to meet the total needs of the world for at least ten years. This batch of PPD has been carefully standardized by tuberculin testing of population groups in different parts of the world. It is used in all of the internationally assisted tuberculosis programs, and it can be made available to any country which may wish to use it. The use of a single batch of tuberculin throughout the world will make international exchange of data on tuberculin testing much more useful.

I should also mention that through the work of WHO's Tuberculosis Research Office a method has been found to stabilize final dilutions of tuberculin to such an extent that they can be used for at least six months after preparation provided they are kept cold and protected from light. This method involves the addition of Tween® 80 to the diluent. The availability of stable ready-made tuberculin dilutions is, of course, a great practical advantage.

On the basis of extensive experience in almost all parts of the world, WHO has recommended a standard tuberculin test—the intradermal test with a single low dose—as well as standard methods for the reading of the reactions and the recording and reporting of results.

With these remarks as an introduction, we come to the question "Can we eliminate tuberculosis as a public health problem?"

In a number of countries, including the United States, tuberculosis mortality and morbidity have in recent years dropped to such low levels

that the responsible public health authorities have been faced with the urgent problem of what, in this situation, should be the tuberculosis program of the country. The countries concerned have all had a tuberculosis control organization for many years, including specialized institutions such as sanatoriums and tuberculosis dispensaries. The problem for their public health administrators is, first of all, whether these special tuberculosis institutions and the other control efforts should be continued and, if so, for how long. There is a very real risk that, as a result of their natural desire to save money, the authorities will allow their allocations to the tuberculosis program to follow the downward trend of the curve for tuberculosis mortality and morbidity in the country. And such a reduction might well benefit the tubercle bacilli more than the people.

To counteract such tendencies, we must define in precise terms what is and what should be the objective of our tuberculosis control program in the present situation. In WHO we define the immediate objective of our tuberculosis program as the elimination of tuberculosis as a public health problem, but we are considering the possibility of having eradication of tuberculosis as our ultimate objective.

Tuberculosis is a public health problem in a community as long as healthy individuals are at a considerable risk of contracting tuberculous disease and, as long as such a risk exists, the special tuberculosis control organization with specialized institutions must be maintained, and perhaps even strengthened. Our aim must be to reduce the risk of contracting tuberculosis to such a low level that we no longer need any specific control measures, but can leave it to the general (nonspecialized) medical and public health services of the community to detect and take care of the few cases of tuberculosis that will continue to occur for a number of years. It is clear that we must be able to define precisely and measure in objective terms when we have reached the point at which we no longer require any specialized institutions or, as a matter of fact, any tuberculosis program. In WHO we have a practical proposal for defining this "target point," when we embark on the surveillance stage of the program leading to eradication.

Our definition must be based on a practical method of measuring the extent of the tuberculosis problem in a community.

In many countries the tuberculosis mortality and morbidity rates up to now have been used as indicators of the time-trend of the tuberculosis problem. It is clear to most people that the advent of effective antituberculous chemotherapy has made the mortality figures almost useless for this purpose. However, even morbidity rates, i.e., the number of newly discovered and reported cases of tuberculosis, are of little help in measuring the extent of the tuberculosis problem. Careful interpretation and extensive knowledge of local conditions are required, as the figures may otherwise be misleading. (One example will illustrate this point: In Glasgow, a city with a population of just over one million, 2,000 new cases of tuberculosis were reported in 1955, and about 1,600 in 1956. In 1957, however, during a five-week period in March–April, no less than 2,565 active cases of pulmonary tuberculosis not previously reported and not previously known came to the knowledge of the health authorities in Glasgow. Was this a serious epidemic of pulmonary tuberculosis? By no means; it was the result of Glasgow's mass X-ray campaign against tuberculosis in which during the five weeks more than 700,000 persons were examined.) While morbidity figures may still have some value in the individual country, they are of practically no value for international comparisons, one reason for this being the lack of an accepted classification of pulmonary tuberculosis. The definition of what should be considered a case of tuberculosis varies from country to country.

Prevalence surveys for tuberculosis in which large, randomly selected samples of the population are subjected to a well-standardized examination for tuberculosis are a useful and practical tool for ascertaining the extent of the tuberculosis problem in countries with a relatively high prevalence of pulmonary tuberculosis (2 to 20 cases per 1,000 adult population). This type of prevalence survey, which is not dependent on any system of reporting of cases, is also useful for international comparison provided standard methods of examination are used; when repeated periodically, it can be used for following the trend of the tuberculosis problem and thus for evaluating the effect of a tuberculosis control program. WHO has put much effort into developing standard methods for prevalence surveys and has carried out such surveys in several countries in Africa. When, as a result of a tuberculosis program, the prevalence of tuberculosis in a country has been reduced to less than 2 cases per 1,000 adult population, such prevalence surveys become impractical: in order to obtain reliable estimates, 50,000 to 100,000 persons have to be examined in each community. At this stage, however, the tuberculin test alone becomes useful.

What we wish to measure is the risk for the healthy individuals in the country of developing tuberculous disease. There is a direct correlation between the risk of developing tuberculous disease and the risk of contracting a tuberculous infec-

tion, and this latter risk can be measured by tuberculin testing. By tuberculin testing a sample of a few thousand children selected at random from the total child population, we can assess fairly precisely the risk of infection existing in a given community.

This index of the risk of disease forms the basis for the definition which we in WHO give of our aim as eliminating tuberculosis as a public health problem. When less than 1 per cent of the children in a community at fourteen years of age are reactors to tuberculin (the WHO standard method for tuberculin testing being used), then the risk in this community of contracting a tuberculous infection, and therefore also of contracting a tuberculous disease, is so small that tuberculosis can no longer be considered a public health problem.

We consider it of the greatest importance that it is possible, by the collection of objective data, to ascertain when the goal (the target point) has been reached or to what degree we have, at a given time, succeeded. We also consider it of the greatest importance that the methods used for this purpose are such that direct international comparisons can be made, and this is one of the reasons why, as I mentioned in my introductory remarks, WHO has devoted so much effort to a complete standardization of tuberculin testing.

Having defined what we understand by the elimination of tuberculosis as a public health problem, we now come to the big question of how we can achieve this aim.

Basically there are today two measures which we can use to influence tuberculosis in a community, both of which are highly effective: BCG vaccination and the use of the antituberculous drugs. The two measures, having quite different modes of action, supplement each other, and both should have their proper place in our tuberculosis program and should be applied correctly.

I wish to emphasize that in WHO we have no doubt about the protective effect of a correctly performed vaccination, and that we consider BCG vaccination just as important for our tuberculosis control program as the use of the antituberculous drugs. I make a point of saying this because recently, following the publication of the results of two controlled trials conducted in the United States, doubts have been raised concerning the protective effect of BCG vaccination. It is important to realize that what I said earlier about the tuberculin test applies also to BCG vaccination. Just as we should stop talking about reactors to tuberculin without specifying the product and the dose of tuberculin used, the method for performing the test and reading the results, so we should stop talking about BCG vaccination as if this were a standard procedure. The vaccine used, the method of application, and several other factors involved are of the greatest importance when considering the effect of the vaccination. In WHO we consider a vaccination successful only if retesting of a sample of the vaccinated subjects shows that a sufficiently high degree of post-vaccination allergy has been induced. From much circumstantial evidence and from the controlled trial of the British Medical Research Council, we know that such a successful vaccination gives a protection of the order of 80 per cent. We also know from data collected in a number of countries in all parts of the world that a sufficiently high degree of post-vaccination tuberculin allergy can be achieved by BCG vaccination when a potent vaccine and the proper method for vaccination are used. The controlled trials carried out in Muscogee County and Puerto Rico, in our opinion, primarily confirm the well-known observation which has also been made by the U.S. Public Health Service in large-scale studies on animals, that a BCG vaccination which does not produce strong and long-lasting post-vaccination allergy cannot be expected to confer a high degree of protection, regardless of whether this unsatisfactory result is due to the use of a weak vaccine or incorrect methods of application.

While we know a good deal about the use of antituberculous drugs for treatment of definite or infectious cases of tuberculosis, and something about their use for preventive treatment of potentially infectious cases, we have so far only very limited knowledge of how best to use these drugs, especially isoniazid, in true prophylaxis. WHO is actively participating in a number of study and research projects in which the problem of prophylactic treatment and chemoprophylaxis are being investigated on a community basis. Time does not permit me to go into any detail concerning this research. Most of it is being carried out in population groups with a high or relatively high prevalence of tuberculosis. But even so, it may well provide us with the answers to a number of questions that are important for the prophylactic use of the antituberculous drugs in population groups with a much lower prevalence.

I should like to mention one WHO-supported study which is still in the discussion and planning stage. This study may be of interest to you partly because it concerns a population group in which the tuberculosis situation is similar to that existing in several states in the United States, and partly because it brings up the type of problem with which we are faced in the last phase of elimi-

nating tuberculosis as a public health problem. The population under study is the total population of Denmark outside the city of Copenhagen. I should like to point out that WHO's participation in this national Danish study project is justified by its importance on an international level; thus, standard methods and procedures will be used throughout.

Before further discussing this study, I must briefly describe the tuberculosis situation as it exists at present in the population to be included in the study. In Denmark, outside the city of Copenhagen, only children in tuberculous families are BCG-vaccinated before school age. At the age of seven years, when the children start going to school, about 2 per cent are found to be reactors to tuberculin. Up to a few years ago children of this age were offered BCG vaccination and almost all nonreactors to tuberculin were vaccinated. This is the reason why I cannot give any figures for the percentage of reactors at the age of fourteen years.

There are two aspects to the study now under discussion: one is the preventive measures to be applied in children and adolescents, and the other the case finding and treatment to be carried out in adults.

It is proposed to postpone BCG vaccination from the time of school entrance to the time of school leaving. At the time of starting school each child will be given the standard tuberculin test. Children found to be reactors to tuberculin will receive isoniazid during the first school year, even if an X-ray examination reveals no sign of tuberculosis. At this time nonreactors will not be BCG-vaccinated. At the beginning of the last normal school year (the children then being about fourteen years of age), a further tuberculin test will be given. All children found to be reactors to tuberculin will be given isoniazid for one year, including those who have already had a course of isoniazid at the age of seven. Children found to be nonreactors to tuberculin will be BCG-vaccinated, so that the total adolescent and adult population will be tuberculin positive.

With regard to the adult population, there are two problems to be studied: mass case finding and treatment of suspects.

It is evident that, in a program directed at elimination of tuberculosis as a public health problem and with the highly effective antituberculous drugs at our disposal today, we cannot be satisfied with diagnosing and treating cases of tuberculosis in people who, because of symptoms, appear for or are referred for specialist examination at the well-equipped and well-staffed tuberculosis dispensaries which exist in every county in Denmark. We wish to reveal and treat the patients before they show any symptoms, and we are prepared to give prophylactic treatment to groups of people who, because of the X-ray picture they show, must be regarded as running a high risk of developing tuberculous disease and therefore can be considered potential cases of tuberculosis. It is important to reveal all such cases, and it follows therefore that mass case-finding programs must be developed.

During recent years in Denmark as in many other countries, systematic examination for tuberculosis of total population groups has largely been confined to so-called "organized" groups such as factory workers, state employees, soldiers, et cetera, and in these groups the examination has been repeated, often after an interval of only one year. It has become evident that this is not the most profitable way of using the mass case-finding units. Community screening must be given more emphasis and applied in such a way as to ensure that a high proportion of people in the older age groups are reached.

One problem which has now to be studied is the best method of organizing and carrying out such community screening for tuberculosis. This method should be practical and economical and at the same time it should ensure that a high proportion of cases of tuberculosis and of potential cases are revealed. The second problem under study is how, on the basis of the examinations and in particular the chest X-ray examination, we can pin-point the groups having a high risk of developing tuberculosis. To do this it is necessary to follow for many years large population groups which have undergone the standard examination for tuberculosis but in which no suspect cases have been treated. The groundwork for these studies has been laid by the Danish Tuberculosis Index which was created in 1951 in cooperation with WHO and which since has received continuing support from WHO. The Danish Tuberculosis Index maintains a card index containing individual records for the 1.5 million people examined in the nation-wide case-finding program of 1950–1952. With these records, which give detailed results of the examinations, all tuberculosis notifications and all death certificates for tuberculosis deaths are matched.

Further studies are needed before we can correctly define the groups of potential cases of pulmonary tuberculosis to which we are prepared to give one year's prophylactic treatment with isoniazid.

This is not the place to go into the details of how this whole project is going to be arranged.

From what I have said I think you will realize, however, that the answers to the questions under study will also be of considerable interest to other countries, including the United States.

And this brings me to the final statement I wish to make.

Possibilities do exist for carrying out successfully programs for the elimination of tuberculosis as a public health problem. However, considerable changes will have to be made in the existing so-called tuberculosis control programs in order to apply on a sufficiently large scale all of the effective measures which are at our disposal today.

During this transition from a program aiming at control of tuberculosis to one aiming at elimination of tuberculosis as a public health problem, it is extremely important to be able to exchange knowledge and experience between countries in an intelligent way. We must pool our knowledge and experience, and there is therefore need for an international organization such as WHO. Further, to be able to undertake this important task successfully, WHO needs the active support and direct cooperation not only of the public health authorities but also of the voluntary organizations in countries such as the United States.

Obituary

Joseph David Aronson
1889–1958

Joseph David Aronson, Professor of Bacteriology at the Henry Phipps Institute for the Study, Treatment and Prevention of Tuberculosis, School of Medicine of the University of Pennsylvania, died while on a field trip to Surinam on October 17, at the age of sixty-nine.

Born in Srednik, Lithuania (then Russia) in 1889, he was brought to this country in his infancy and became an American citizen at the age of seven through his father's naturalization. His early years were spent in New York and Philadelphia, and it was at the old Medico-Chirurgical College of Philadelphia that he took his M.D. degree in 1908 at the age of nineteen. After an eighteen-month rotating internship at the Blockley (now the Philadelphia General) Hospital, Dr. Aronson was Instructor in Surgical Pathology at the Medico-Chirurgical College for one year, then Professor of Pathology and Bacteriology at the University of Arkansas from 1911–1914. Returning to Philadelphia, he spent several years as assistant bacteriologist at the Board of Health Laboratory, then a year as Director of the City Hospital Laboratory, Greenville, South Carolina, before joining the Henry Phipps Institute in 1920.

First as a Captain, later as a Colonel, in the Army Medical Corps, Dr. Aronson served overseas in both world wars. His duties as Laboratory Officer took him in 1918 and again in 1944 to the Pasteur Institute in Paris, where he became a confirmed francophile and a devoted friend of A. Boquet and L. Nègre.

The spread of infection, allergy, immunity,

virulence—those were the matters that Joseph Aronson found of compelling and lifelong interest. He sought answers to these problems through work with tuberculin with acid-fast organisms, particularly tubercle bacilli and BCG, and with leprosy. By-products from his field observations included, among others, a brief history of disease in Alaska (written while snowbound there), and the establishment of the prevalence of coccidioidomycosis in Southern Arizona and the significance of that infection as a cause of pulmonary calcification. In one of his early papers, published with Paul A. Lewis in 1923 in the AMERICAN REVIEW OF TUBERCULOSIS, his scientific curiosity, his appreciation of basic principles, his drive for better methods were clearly revealed: "Incidentally, however, we became impressed with the belief that this method might afford the basis for an improvement in the current procedure of tuberculin standardization." His interest in tuberculin led to pioneer work in tissue culture studies and the discovery of the cytotoxic reaction of tuberculin on tissue cultured from tuberculous animals. It also led in the early 1930's to extensive field studies on the prevalence of tuberculous infection in rural districts of the South—"astonishing high rates in those days"—and rural regions in Michigan for comparison.

Joe Aronson was at home with many types of tubercle bacilli. As Paul Lewis once said, he had a special "kunst" for isolating acid-fast organisms. Recognized as the American authority on tuberculosis in cold-blooded animals, his *Mycobacterium marinum* (Aronson) from salt water fish and *Mycobacterium thamnopheos* (Aronson) from garter snakes are listed in Bergey's *Manual of Determinative Bacteriology*. He worked with Eugene Opie on tubercle bacilli in human lesions and with veterinary pathologists on differentiating human and bovine bacilli. Leprosy bacilli also claimed his attention for many years, and at the time of his death he was collecting leprosy tissues in the hope of culturing leprosy bacilli in cold-blooded animals.

His first paper on BCG was published with A. M. Dannenberg in the *American Journal of Diseases of Children* in 1935. That same year he started his world-renowned study of BCG vaccination in American Indians living in Arizona, Wyoming, the Dakotas, and Alaska. This was the first scientific attempt in any country to evaluate the effectiveness of BCG by including a randomly selected unvaccinated control group—a courageous and almost revolutionary departure from methods considered acceptable at that time. Of the next five years his wife wrote: "Come hell, or highwater, sandstorms or ice he made the rounds once a year. His Indian name among the Sioux meant 'chain lightening'!" And for more than twenty years he continued to follow the vaccinated and control groups—a remarkable feat calling for the indefatigability, persistence, and dedication that characterized Joe Aronson's professional career.

The Alvarenga Prize, awarded annually by the College of Physicians of Philadelphia "to the author of the best published or unpublished work, on any medical subject," was awarded to Dr. Aronson in 1947. Two years later, he received the Distinguished Service Award from the Department of the Interior "For his eminent career and outstanding contribution to the public service." In connection with the award, he received a gold medal and his name was inscribed in the lobby of the Department.

Joe Aronson was a hard worker, putting tremendous energy and enthusiasm into the many stimulating tasks he was continually setting for himself. He thrived on new places, new people and new ideas; but even alone on an island he would have found absorbing problems and set about solving them. He was productive, contributing a wealth of information on basic problems in tuberculosis and allied fields. He had the curiosity, the integrity, the self-discipline, and above all the undeniable inner drive so necessary for real scientific work. His was a life which successfully upheld and perpetuated the finest traditions of medical science.

CARROLL E. PALMER

NOTICES

The following dates and location of the next four annual meetings of the National Tuberculosis Association and the American Trudeau Society have been selected and approved:

1959—Chicago, Illinois—May 24–29
1960—Los Angeles, California—May 15–20
1961—Cincinnati, Ohio—May 21–26
1962—Miami Beach, Florida—May 20–25

AMERICAN COLLEGE OF CHEST PHYSICIANS—25TH ANNUAL MEETING

The American College of Chest Physicians will hold its Silver Anniversary meeting at the Ambassador Hotel, Atlantic City, June 3–7, 1959. The scientific program will include prominent speakers on all aspects of heart and lung diseases. In addition to formal presentations, there will be a number of symposia, round table luncheon discussions, postgraduate seminars, and motion pictures.

Fireside Conferences, inaugurated in 1955, will feature more than 60 experts in chest disease, leading discussions on topics of current interest.

Examinations for Fellowship in the College will be held on Thursday, June 4, and on Thursday evening, more than 200 new Fellows will receive their certificates of Fellowship at the Convocation. The Presidents' Banquet and annual dance will take place on Saturday, June 6.

It is expected that a number of physicians from other countries will attend the 25th Annual Meeting.

Dr. Donald R. McKay, Buffalo, New York, President of the College, has announced that to commemorate the Silver Anniversary, the College is publishing a history of the growth of the organization since its first meeting in 1935.

AMERICAN COLLEGE OF RADIOLOGY TO PRODUCE RADIATION PROTECTION MOTION PICTURE FOR PHYSICIANS

Chicago—A motion picture illustrating the medical aspects of radiation, including protective measures in diagnostic radiologic examinations, will be distributed soon by the American College of Radiology to the nation's physicians.

Supervising production of the 16-mm., half-hour color film will be a special committee of the American College of Radiology under the chairmanship of Wendell G. Scott, M.D., professor of clinical radiology, Washington University School of Medicine, St. Louis, Missouri.

Also members of the supervising committee are Richard H. Chamberlain, M.D., professor of radiology, University of Pennsylvania School of Medicine, Philadelphia, Pa.; Philip J. Hodes, M.D., professor of radiology, Jefferson Medical College, Philadelphia, Pa.; Ted. F. Leigh, M.D., professor of radiology, Emory University School of Medicine, Emory University, Ga., and Donald Chadwick, M.D., and Arthur Wolff, D.V.M., both of the United States Public Health Service, Washington, D. C.

Mr. Stephen Schwartz, associated with the Medical Radio and Television Institute, New York City, has been named producer for the film.

The motion picture has been financed through a Rockefeller Foundation grant and College funds.

Premiere showing of the film is June 9, 1959, at the meeting of the American Medical Association in Atlantic City, N. J. Following the premiere, prints will be released immediately for distribution among the medical profession for showings at scientific programs, medical conventions, medical society meetings, hospital medical staff meetings, and hospital association conventions.